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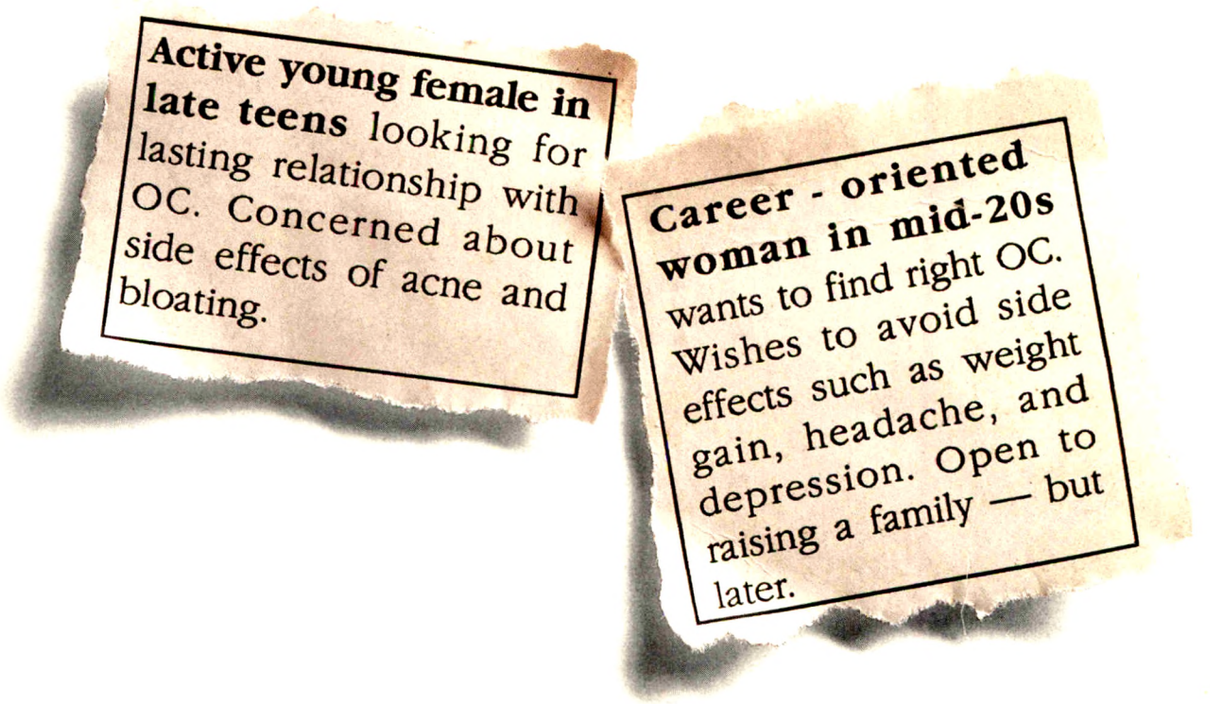
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Active young female in late teens looking for lasting relationship with OC. Concerned about side effects of acne and bloating.
Career - oriented woman in mid-20s wants to find right OC. Wishes to avoid side effects such as weight gain, headache, and depression. Open to raising a family — but later.

Nonsmoking, healthy woman in late-30s wishes to restart relationship with oral contraceptive. Low metabolic and hormonal impact preferred.

A personalized approach to oral contraceptives.

Because Tri-Norinyl is uniquely phased for all phases of a woman's reproductive life, it's ideal for a wide range of patient types:

Older, nonsmoking patients restarting contraception, because Tri-Norinyl is the only multiphasic with low-impact norethindrone that *lowers* progestin in late cycle to minimize overall hormonal and metabolic impact.

Career-oriented patients delaying childbearing, because reduced progestin helps minimize side effects that can disrupt daily life, such as weight gain, headache, and depression.

Younger patients just starting contraception, because low-impact norethindrone lowers the risk of acne and bloating, and is increased at mid-cycle, to provide enhanced endometrial support when breakthrough bleeding is most likely to occur.

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

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Committed to the OB/GYN and women's healthcare

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Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see next page for brief summary of prescribing information.

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(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

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Uniquely phased for all phases of a woman's reproductive life

ORAL CONTRACEPTIVE AGENTS: BRIEF SUMMARY

DESCRIPTION

TRI-NORINYL 21-DAY Tablets provide an oral contraceptive regimen of 7 blue tablets followed by 9 yellow-green tablets and 5 more blue tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg and each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg.

TRI-NORINYL 28-DAY Tablets provide a continuous oral contraceptive regimen of 7 blue tablets, 9 yellow-green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg, each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert ingredients.

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions: Thrombophlebitis or thromboembolic disorders • A past history of deep vein thrombophlebitis or thromboembolic disorders • Cerebral vascular or coronary artery disease • Known or suspected carcinoma of the breast • Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia • Undiagnosed abnormal genital bleeding • Cholestatic jaundice of pregnancy or jaundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors • Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholesterolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estrogen. The effects of long-term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the *ratio* of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide not only a measure of the relative risk but a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

a. Myocardial infarction

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS**, section 9). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2- to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970's—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral contraceptive use to women who do not have the various risk factors listed in this labeling.

Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular disease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (> 8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users.

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0.075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed earlier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The incidence of risk also was reported to increase with continued use and among older women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens.

Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especiall when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the ini ation or reinstitution of oral contraceptives and at least annually during use of oral co traceptives. These physical examinations should include special reference to bloo pressure, breasts, abdomen and pelvic organs, including cervical cytology and rel vant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vagin bleeding, appropriate diagnostic measures should be conducted to rule out mali nancy. Women with a strong family history of breast cancer or who have breast nodule should be monitored with particular care.

2. LIPID DISORDERS

Women who are being treated for hyperlipidemias should be followed closely if the elect to use oral contraceptives. Some progestogens may elevate LDL levels and ma render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication shou be discontinued. Steroid hormones may be poorly metabolized in patients wi impaired liver function.

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be pr scribed with caution, and only with careful monitoring, in patients with condition which might be aggravated by fluid retention.

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug disco tinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance shoul be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstru irregularities have been associated with concomitant use of rifampin. A similar asso ation though less marked, has been suggested with barbiturates, phenylbutazon phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected b oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3 increased norepinephrine-induced platelet aggregability.

b. Increased thyroid binding globulin (TBG) leading to increased circulating total th roid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioi munoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T concentration is unaltered.

c. Other binding proteins may be elevated in serum.

d. Sex steroid binding globulins are increased and result in elevated levels of total circ lating sex steroids and corticoids; however, free or biologically active levels remai unchanged.

e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum folate levels may be depressed by oral contraceptive therapy. This may be clinical significance if a woman becomes pregnant shortly after discontinuing or contraceptives.

9. CARCINOGENESIS

See **WARNINGS** section.

10. PREGNANCY

Pregnancy Category X. See **CONTRAINDICATIONS** and **WARNINGS** sections.

11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursin mothers and a few adverse effects on the child have been reported, including jaundic and breast enlargement. In addition, oral contraceptives given in the postpartu period may interfere with lactation by decreasing the quantity and quality of brea milk. If possible, the nursing mother should be advised not to use oral contraceptive but to use other forms of contraception until she has completely weaned her child.

INFORMATION FOR THE PATIENT

See **PATIENT LABELING.**

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated wit the use of oral contraceptives (see **WARNINGS** section): Thrombophlebitis • Arteri thromboembolism • Pulmonary embolism • Myocardial infarction • Cerebral hemo rhage • Cerebral thrombosis • Hypertension • Gallbladder disease • Hepatic adenoma carcinomas or benign liver tumors

There is evidence of an association between the following conditions and the use oral contraceptives, although additional confirmatory studies are needed: Mese teric thrombosis • Retinal thrombosis

The following adverse reactions have been reported in patients receiving oral contr ceptives and are believed to be drug-related: Nausea • Vomiting • Gastrointestin symptoms (such as abdominal cramps and bloating) • Breakthrough bleeding • Spo ting • Change in menstrual flow • Amenorrhea • Temporary infertility after discontinu tion of treatment • Edema • Melasma which may persist • Breast changes: tenderne enlargement, secretion • Change in weight (increase or decrease) • Change in cervic erosion and secretion • Diminution in lactation when given immediately postpartum Cholestatic jaundice • Migraine • Rash (allergic) • Mental depression • Reduced tol ance to carbohydrates • Vaginal candidiasis • Change in corneal curvature (steepe ing) • Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contraceptives an the association has been neither confirmed nor refuted: Pre-menstrual syndrome Cataracts • Changes in appetite • Cystitis-like syndrome • Headache • Nervousness Dizziness • Hirsutism • Loss of scalp hair • Erythema multiforme • Erythema nodosu • Hemorrhagic eruption • Vaginitis • Porphyria • Impaired renal function • Hemolyt uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses oral contraceptives by young children. Overdosage may cause nausea, and withdraw bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contrace tives are supported by epidemiological studies which largely utilized oral contrace tive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol 0.05 mg of mestranol.

Effects on menses: • Increased menstrual cycle regularity • Decreased blood los and decreased incidence of iron deficiency anemia • Decreased incidence dysmenorrhea

Effects related to inhibition of ovulation: • Decreased incidence of functional ovari cysts • Decreased incidence of ectopic pregnancies

Effects from long-term use: • Decreased incidence of fibroadenomas and fibrocyst disease of the breast • Decreased incidence of acute pelvic inflammatory disease Decreased incidence of endometrial cancer • Decreased incidence of ovarian canc

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Columbus, Ohio, and Omaha, Nebraska

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Durham, North Carolina

Overexpression of the HER-2/*neu* oncogene in endometrial cancer is associated with the presence of metastatic disease and poor survival.

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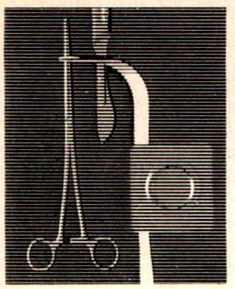
(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

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Count on

CEFOTAN® IM/IV

(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary tract infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).
Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli.*
Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli.*
Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).
Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).
Bone and joint infections caused by *Staphylococcus aureus.**
*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.
NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.

PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.
INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.
DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.
CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.
USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.
USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.
PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).
Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).
Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).
Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).
Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.
IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

Females: 0.9 × value for males

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.

1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

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Wilmington, Delaware 19897 USA

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63985-01

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Roberto Matorras, MD, PhD, Jose M. Ariceta, MD, Angel Rementeria, MD, Jesús Corral, MD, Gloria Gutierrez de Terán, RN, Javier Diez, MD, Francisco Montoya, MD, PhD, and Francisco J. Rodriguez-Escudero, MD, PhD
Bilbao, Spain

Human immunodeficiency virus–positive women were at high risk of human papillomavirus infection, and among them human papillomavirus–associated lesions had a more resistant behavior.

Endometriosis associated with massive ascites and absence of pelvic peritoneum 45
R. Stan Williams, MD, and Rebecca Wagaman, MD
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Seth Granberg, MD, Mats Wikland, MD, Bengt Karlsson, MD, Anders Norström, MD, and Lars-Gösta Friberg, MD
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Mitchel S. Hoffman, MD, Lorraine W. Gordy, CT, and Denis Cavanagh, MD
Tampa, Florida

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Low level of maternal serum α-fetoprotein: Its associated anxiety and the effects of genetic counseling 54
Kathy L. Keenan, MS, Deborah Basso, MD, John Goldkrand, MD, and William J. Butler, MD
Albany, New York

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Successful antepartum treatment of listeriosis 57
Charles Kalstone, MD
Miami, Florida

Recognizing and treating the pregnant patient with listeriosis can save her fetus.

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Mark A. Klebanoff, MD, MPH, Patricia H. Shiono, PhD, Joseph V. Selby, MD, MPH, Alan I. Trachtenberg, MD, MPH, and Barry I. Graubard, MA
Bethesda, Maryland, and Oakland, California

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Atlanta, Georgia, Newark, New Jersey, Baltimore, Maryland, and West Plains, Missouri

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Ottawa, Ontario, Canada

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Durham, North Carolina

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Phosphorus 31 magnetic resonance spectroscopy of human placenta and quantitation with perchloric acid extracts 80
Helen H. Kay, MD, John D. Gordon, MD, Anthony A. Ribeiro, PhD, and Leonard D. Spicer, PhD
Durham, North Carolina

Phosphorus 31 nuclear magnetic resonance spectroscopy of human placental tissue is reported and promises to be an exciting method for placental metabolism studies.

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To be continued...



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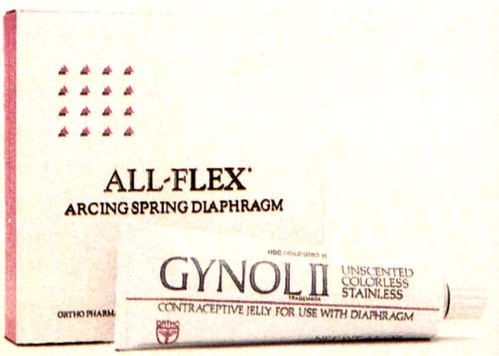
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‡Data on file, Ortho Pharmaceutical Corporation.

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ORTHO

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Giovanni Battista Candiani, MD, Paolo Vercellini, MD, Luigi Fedele, MD, Salvatore Garsia, MD, Diana Brioschi, MD, and Laura Villa, MD
Milan, Italy

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Winnipeg, Manitoba, Canada

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Chapel Hill, North Carolina

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Pittsburgh, Pennsylvania

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Santa Barbara, California, Bethesda, Maryland, Chicago, Illinois, Seattle, Washington, and Pittsburgh, Pennsylvania

Adjusted third-trimester nonfasting glucose levels and glycosylated hemoglobin levels in insulin-dependent diabetic women predict the risk of macrosomia.

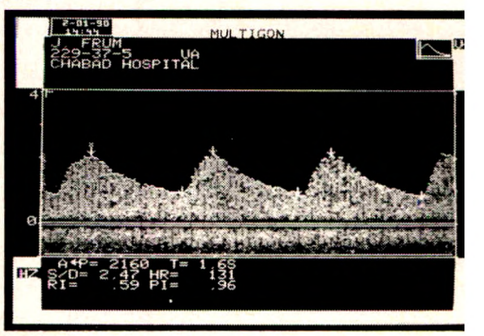
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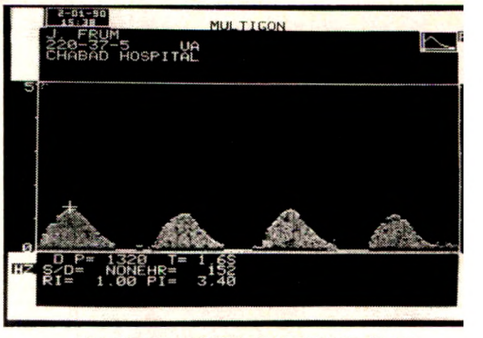


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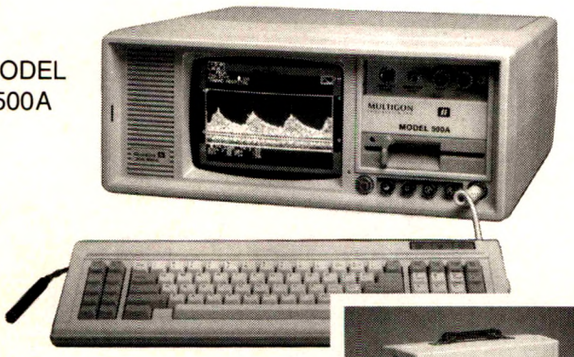


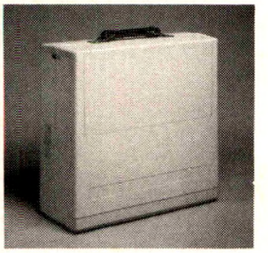
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Montreal, Quebec, Canada

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Burlington and Hamilton, Ontario, Canada

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Michiel C. van der Pol, MD, Mijna Hadders-Algra, MD, Henk J. Huisjes, MD, and Bert C.L. Touwen, MD
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New Haven, Connecticut

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Stanley A. Gall, MD, Lisa Constantine, RN, and Doris Koukol, RN
Louisville, Kentucky

The interferons used in this study were effective in the treatment of persistent human papillomavirus disease of the vulva and enhanced subsequent adjunctive therapies.

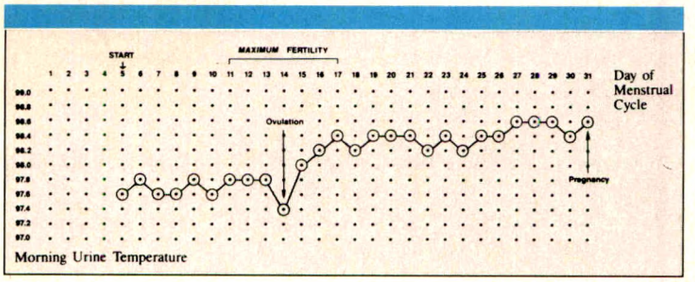
Cross-sectional analysis of triplet birth weight 135
J. Stephen Jones, MD, Roger B. Newman, MD, and M. Clinton Miller, PhD
Charleston, South Carolina

Triplet growth appears distinctly linear between 22 and 38 weeks' gestation with mean intratriplet discordance found to be double that of twin pregnancies.

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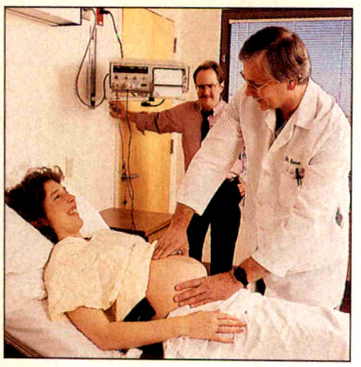
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2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY. Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.
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INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risk, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy; changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.

Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

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1. Nilas L, Christiansen C, Rødbro P: Calcium supplementation and postmenopausal bone loss. *Br Med J* 1984; 289:1103-1106. **2.** Riggs BL: Research Directions in Osteoporosis. Scientific Workshop. National Institutes of Health. February 9-11, 1987. **3.** Kiel DP, Felson DT, Anderson JJ, et al: Hip fracture and the use of estrogens in postmenopausal women. *N Engl J Med* 1987;317:1169-1174. **4.** Christiansen C, Christensen MS, Transbøl I: Bone mass in postmenopausal women after withdrawal of oestrogen/gestagen replacement therapy. *Lancet* 1981;1:459-461.

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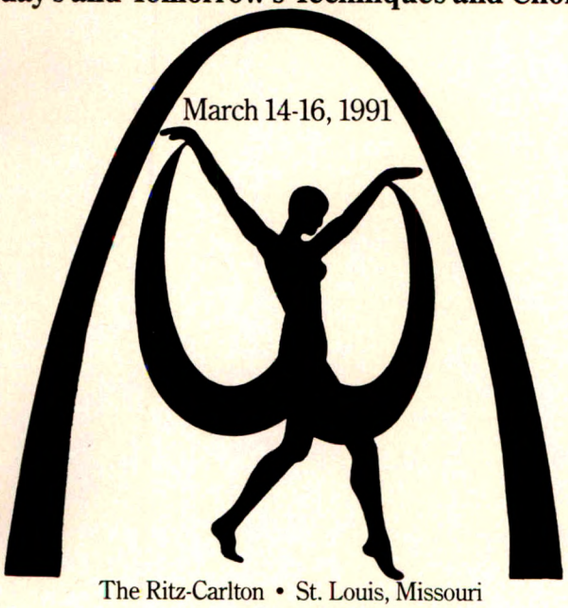
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The Third International Vaginal Surgery Conference

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PROGRAM

THURSDAY, MARCH 14, 1991

7:00 A.M.: Registration
7:45 A.M.: Welcome/S. Robert Kovac, M.D.
7:50 A.M.: Stephen H. Cruikshank, M.D. (Scientific Moderator)
8:00 A.M.: L. Russell Malinak, M.D. *"The Decline of Vaginal Hysterectomy"*
8:35 A.M.: Sheldon A. Weinstein, M.D. *"Vaginal Hysterectomy and Oophorectomy"*
9:10 A.M.: Javier F. Magrina, M.D. *"The Use of Self-retaining Retraction in Vaginal Surgery"*
9:45 A.M.: Break
10:15 A.M.: Richard J. Gimpelson, M.D. *"Operative Office Hysteroscopy"*
10:35 A.M.: M. Leon Tancer, M.D. *"The Martius Operation"*
11:05 A.M.: J. Andrew Fantl, M.D. *"Bladder Drainage in Pelvic Surgery"*
11:25 A.M.: Lane J. Mercer, M.D. *"The Use of Antibiotics for Vaginal Surgery"*
Noon: Question and Answer Period
12:30 P.M.: Lunch
1:30 P.M.: Robert F. Porges, M.D. *"Vaginal Plastic Repair"*
2:05 P.M.: Wayne F. Baden, M.D. *"Management of Anterior Vaginal Defects: Urethrocele, Anatomic Urinary Incontinence and Cystocele"*
2:40 P.M.: M. Leon Tancer, M.D. *"Vaginal Hysterectomy the Suture Technique"*
3:10 P.M.: Break
3:30 P.M.: G. Rodney Meeks, M.D. *"Vaginal Hysterectomy in a Gynecologic Training Program"*
4:05 P.M.: Round Table
Moderators: S. Robert Kovac, M.D.—Stephen H. Cruikshank, M.D.
"Complications of Vaginal Surgery"
Javier F. Magrina, M.D. — G. Rodney Meeks, M.D. — M. Leon Tancer, M.D.
Sheldon A. Weinstein, M.D. — Robert F. Porges, M.D. — L. Russell Malinak, M.D.
4:45 P.M.: Question and Answer Period
5:15 P.M.: Adjourn
Wine and Cheese Reception/Exhibit Hall

FRIDAY, MARCH 15, 1991

7:30 A.M.: Update/Stephen H. Cruikshank, M.D.
7:45 A.M.: Wayne F. Baden, M.D. *"Is a Universal Language for Vaginal Relaxation Desirable? Possible? Available?"*
8:25 A.M.: L. Russell Malinak, M.D. *"Laparoscopy and Vaginal Hysterectomy"*
9:00 A.M.: S. Robert Kovac, M.D. *"Abdominal Hysterectomy for Fibroids—The Dying Art"*
9:45 A.M.: Break
10:00 A.M.: Stephen H. Cruikshank, M.D. *"Sacrospinous Fixation SHOULD it be done at the Time of Vaginal Hysterectomy?"*
10:35 A.M.: John O. L. DeLancey, M.D. *"Functional Anatomy of the Pelvic Floor"*
11:05 A.M.: Robert F. Porges, M.D. *"Management of Giant Enterocele"*
11:40 A.M.: Question and Answer Period
Noon: Lunch
1:00 P.M.: *"Joseph H. Pratt, M.D. Vaginal Surgeon's Award"*
Presented by: S. Robert Kovac, M.D.
Presented to: Joseph H. Pratt, M.D.
1:10 P.M.: Javier F. Magrina, M.D. *"Vaginal Vault Repair—Mayo Culdoplasty"*
1:45 P.M.: Richard J. Gimpelson, M.D. *"Hysteroscopic Myomectomy"*
2:05 P.M.: Sheldon A. Weinstein, M.D. *"Vaginal Surgery Tricks"*
2:45 P.M.: Lane J. Mercer, M.D. *"Treatment of Severe Prolapse with Colpocleisis"*
3:15 P.M.: Break
3:45 P.M.: G. Rodney Meeks, M.D. *"Vaginal Hysterectomy for Enlarged Uteri"*
4:20 P.M.: Question and Answer Period
4:45 P.M.: Adjourn
6:30 P.M.: Social Dinner/Dance

SATURDAY, MARCH 16, 1991

7:30 A.M.: Update/S. Robert Kovac, M.D.
7:40 A.M.: Robert F. Porges, M.D. *"Anatomy and Physiology of Pelvic Support"*
8:00 A.M.: J. Andrew Fantl, M.D. *"Lower Urinary Tract in Severe Prolapse"*
8:20 A.M.: M. Leon Tancer, M.D. *"Vesicovaginal Fistula and Surgical Management of Operative Cystotomy"*
8:50 A.M.: Lane J. Mercer, M.D. *"Diseases and Surgery of the Female Urethra"*
9:15 A.M.: Break
9:30 A.M.: John O. L. Delancey, M.D. *"Anatomy of Stress Incontinence and the Paravaginal Repair"*
10:05 A.M.: Round Table
Moderators: S. Robert Kovac, M.D.—Stephen H. Cruikshank, M.D.
"Who Should Manage Urinary Incontinence in Women?"
John O. L. DeLancey, M.D. — Robert F. Porges, M.D. — Lane J. Mercer, M.D.
J. Andrew Fantl, M.D. — Wayne F. Baden, M.D. — M. Leon Tancer, M.D.
10:45 A.M.: Question and Answer Period
11:00 A.M.: Adjourn

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1. **Dr. Zuspan**—manuscripts from the eastern United States and from England, Israel, and Italy, manuscripts written for Clinical Opinion and Current Development, Letters to the Editors, and all manuscripts presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation. *Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. Donald R. Coustan, Department of Obstetrics and Gynecology, Women and Infants Hospital of Rhode Island, 101 Dudley St., Providence, RI 02905.*
2. **Dr. Quilligan**—manuscripts from states west of Pennsylvania, West Virginia, Kentucky, Tennessee, and Mississippi and from Hawaii, Alaska, Canada, and abroad (except England, Israel, and Italy). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. *Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.*

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Checklist. The checklist that appears with the copyright statement in each issue of the JOURNAL must be fully completed by the author(s) and sent with each submitted manuscript. A more complete description of each item that must be checked is provided under the appropriate heading in the Information for Authors, which is published in each issue of the JOURNAL. *If the checklist is not completed in full, the deficiencies will be noted, and the form, as well as the manuscript when necessary, will be returned to the author for completion.*

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Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

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In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

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In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare

Estimating length of manuscripts

With the exception of Current Development articles, the length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

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CHECKLIST

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—The original and two copies of the manuscript are submitted.
—The appropriate signed completed checklist and assignment of copyright signed by **all** authors accompany the manuscript.
—Three sets of unmounted glossy prints for regular figures are submitted, properly numbered and labeled on the back of each print.
—Three sets of original computer-generated figures printed on heavy coated paper with either a glossy or dull finish, unmounted, each one on a page, and properly labeled on the back are submitted. None are dot matrix or photographic prints.
—Human experimentation has been approved by the local institution as stated in the Methods section.
—Guidelines for the care and use of animals approved by the institution have been followed as indicated in the Methods section.
—The covering letter with essential information is with the manuscript.
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—All pages are numbered in the following order: title page, condensation, abstract, body of the text, acknowledgments only of persons who have made substantive contributions to the study, references, legends, and tables.
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—Names and addresses of three individuals are enclosed.

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—Three to five key words or short phrases are placed beneath the abstract on page 3.

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—Tables are cited in sequence in the text.

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—Original transparencies of **color photographs** appropriately numbered and identified are submitted along with two sets of unmounted prints on glossy paper, numbered and identified on the back.

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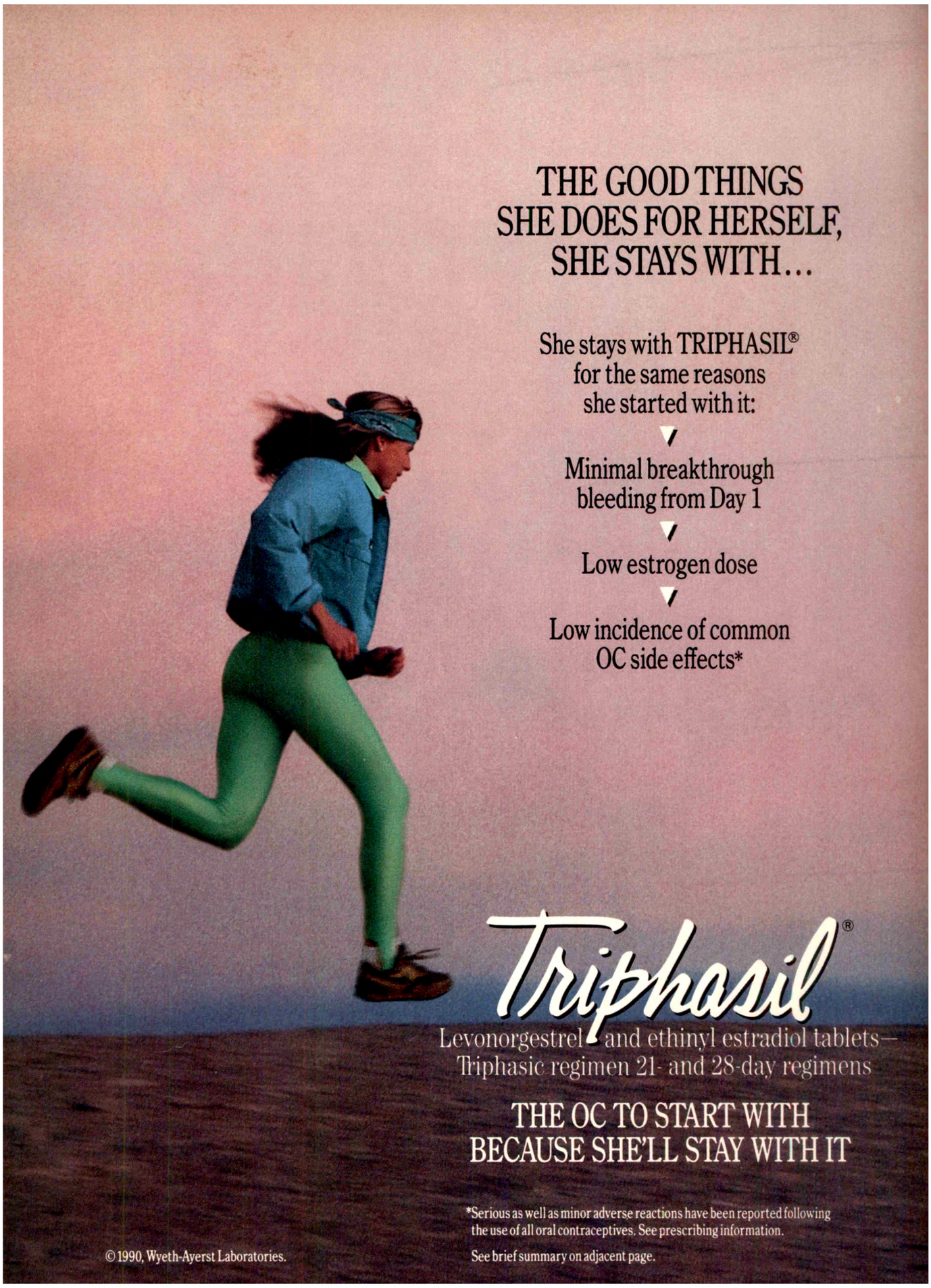
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THE OC TO START WITH
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*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. See prescribing information.
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Triphasil®

Levonorgestrel and ethinyl estradiol tablets—
Triphasic regimen 21- and 28-day regimens

THE OC TO START WITH, BECAUSE SHE'LL STAY WITH IT

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TRIPHASIL® — 6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen) — Triphasic regimen.

Indications and Usage — TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications — OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thrombo-embolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners pre-scribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS — In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI) — An increased risk of MI associated with the use of OC's has been reported, con-firming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE — In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES — A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34 — 5/100,000; ages 35-44 — 33/100,000; ages 45-49 — 140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS — Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions* — There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma* — Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic mea-sures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors* — Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring* — Use of female sex hormones — both estrogenic and progestational agents — during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease* — Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects* — Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure* — Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache* — Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities* — Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy* — Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding* — OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions — GENERAL — 1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear. 2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related. 4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined. 8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient — See Patient Package Labeling.

Drug Interactions — Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis — See Warnings section for information on carcinogenesis.

Pregnancy — Category X. See Contraindications, Warnings.

Nursing Mothers — See Warnings.

Adverse Reactions — An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose — Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration — For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.

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EDITORIAL

A change in the masthead

Many of you may have noticed significant changes on the cover of this issue of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY. John Brewer, after 32 years of dedicated service to the JOURNAL as Editor in Chief, has requested that he become Editor Emeritus. We, and the Publisher, tried to convince John to postpone this decision for a few years, but anyone who knows John realizes that after he has thought through a difficult decision, it is almost impossible to get him to change his mind. We have prospered from and enjoyed tremendously our 20-year association with John as our JOURNAL boss and mentor role model. He has been a personal friend who has given us tips on everything from life to golf, each one given with a deep background of experience and expertise, and always with our best interests in mind. John has also been a personal friend to each of you who reads the JOURNAL by insisting on the most rigorous standards for the authors, the Editors, and the Publisher: every *i* dotted and every *t* crossed, all for the benefit of the reader. He has simultaneously embraced change when he felt it would make the JOURNAL stronger: peer review of all articles, development of the Clinical Opinion section, and addition of the short abstract with the title in the table of contents to name just a few. If you think this guiding hand will be completely lost, rest assured it will not. John has agreed to attend our editorial meetings and for this we, the Editors and Publisher, know the reading public will be grateful.

You will also notice some new names on the cover. Drs. Gabbe, Garite, Kim, and Manetta have agreed to become Associate Editors of the JOURNAL. We were very pleased that such outstanding people in the specialty with special expertise in maternal-fetal medicine, endocrinology-infertility, and gynecologic oncology would join us. They add further depth to the JOURNAL staff, which should assure both reader and author that we are constantly trying to improve the JOURNAL. Having our Associate Editors in our own institutions should speed the flow of articles and allow us to delete one JOURNAL office. This reorganization is not accomplished without loss. Dr. Gerbie, who has been an important part of the JOURNAL as an Associate Editor in Dr. Brewer's office, will no longer serve in that capacity. We thank him for all his contributions in the past and know that we can call on him for advice in the future. Sandra Fernandez, Senior Editorial Assistant for the JOURNAL, will be assuming new duties at Northwestern University School of Medicine. Her efficient office management and her expertise in working with authors, reviewers, and Mosby–Year Book personnel have contributed immensely to the success of the JOURNAL. Fortunately for us she has agreed to help if the need arises. Her vast knowledge about the workings of the JOURNAL ensures that we will be consulting her in the future.

Change is inevitable. It is always accompanied by regrets at the good that is lost; however, it can make us work harder to live up to the standards John Brewer has set for the JOURNAL. The Editors and Associate Editors assure you that this will be our goal.

The Editors

Clinical Opinion

Blood pressure self-monitoring during pregnancy: Practical considerations

Frederick P. Zuspan, MD,[a] and William F. Rayburn, MD[b]
Columbus, Ohio, and Omaha, Nebraska

Portable electronic and mechanical aneroid instruments for blood pressure self-monitoring are available, inexpensive, and reasonably accurate. Instructing the patient at risk for borderline, mild hypertension or chronic hypertension during pregnancy on self-monitoring of blood pressure has advantages. Combined with conventional office measurements, many daily recordings give an accurate picture of an individual's dynamic blood pressure. Lower blood pressures commonly found outside the office are likely attributable to an increased awareness, less anxiety, and attention by the patient to nonpharmacologic factors. Such persons may well avoid antihypertensive therapy or antepartum hospitalization. This information provides a strong incentive to undertake and adhere to an appropriate plan of treatment for those patients whose hypertension is confirmed by blood pressure self-monitoring technique. We consider blood pressure self-measurement to complement other reported monitoring techniques for active patient participation at home or in the work setting. It also permits an early evaluation of blood pressure alterations to rule out preeclampsia, especially in the pregnant patient with chronic essential hypertension. (AM J OBSTET GYNECOL 1991;164:2-6.)

Key words: Blood pressure self-monitoring, pregnancy, hypertension, preeclampsia

Most health care personnel tend to think of hypertension during pregnancy as a fixed entity, but blood pressures actually show daily variations regardless of physical and mental activities. "White coat" hypertension is a specific phenomenon—possibly a conditioned response—that does not necessarily indicate excessive blood pressure reactivity at other times.[1] Thus even confirming the diagnosis of hypertension during pregnancy by verifying high blood pressure values during repeated office visits is usually inadequate. Blood pressure self-determinations will often answer these questions.

Blood pressure self-monitoring has been proposed for the evaluation and treatment of pregnant patients at risk for or presumed to have borderline, mild, or overt hypertension.[2] Recordings of blood pressure over the day and night are "dynamic" rather than "static" and, combined with conventional office measurements, may provide the degree of accuracy necessary to plan management. This manuscript will discuss our clinical opinions about the impact of blood pressure self-monitoring on decision-making for management and eventual perinatal outcomes. We will also describe the equipment and procedures and identify patients who would benefit most from such monitoring.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The Ohio State University College of Medicine,[a] and the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Nebraska College of Medicine.[b]
Reprint requests: Frederick P. Zuspan, MD, Department of Obstetrics and Gynecology, 5th Floor, Means Hall, 1654 Upham Dr., Columbus, OH 43210-1228.
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Concept of blood pressure self-monitoring

Studies of whole-day blood pressure monitoring have used portable intraarterial direct surveillance systems in nonpregnant adults.[3, 4] Although this approach provides accurate and continuous measurements, its invasive and costly nature makes it impractical for routine clinical applications. The use of blood pressure self-monitoring devices is more practical. Recordings at home, work, or elsewhere allow patients and their families to actively participate in the treatment program. Those patients receiving antihypertensive therapy are provided an instant visual evidence of blood pressure change and their response to therapy. This instant feedback of information should enhance compliance with the prescribed treatment programs.

Measurements outside the obstetrician's office may aid in making treatment decisions for pregnant patients with hypertension and in evaluating or adjusting ther-

apy.[2] Blood pressure self-measurements may be particularly useful for patients on a treatment program that includes nonpharmacologic measures. It may also lead to simplification of antihypertensive treatment, possibly resulting in a reduction of drug doses, fewer side effects, and increased compliance. Once the "goal" blood pressure is achieved, maintained, and understood by the patient, the self-recordings may permit longer intervals between office visits. We normally would like to examine pregnant patients with mild chronic hypertension (diastolic pressure >90 mm Hg) at least every 2 weeks until the thirtieth week of gestation and then on a weekly basis.

Investigations during pregnancy

For more than 12 years, we have encouraged our pregnant patients to measure their own blood pressures and have found the practice satisfactory in monitoring those with hypertension from any cause. Blood pressure values obtained at home have been lower than clinic measures in 52% of the cases. Averaged home measures have been equivalent (within 5 mm Hg) to clinic measures in only 28% of clinic visits.[2] Pressures persistently higher at home than in the clinic have been extremely rare. These facts should cause concern in evaluating data in publications that do not utilize blood pressure self-monitoring because many patients who probably do not need it will be placed on a regimen of antihypertensive medication. These observations have been confirmed with a telemetric technique to transmit blood pressures from home to clinic.[5] Self-recordings of blood pressure have improved the patient's awareness and provide additional information with which to formulate management decisions. Lower blood pressures at home are expected to be associated with bed rest, positional change, and the averaging of daily recordings, rather than the customary one or two values obtained during weekly or biweekly office visits.

We feel that pregnant women with hypertension may be managed at home if blood pressures are borderline or mildly elevated in the clinic but normal at home. An investigation conducted at our clinics during a 2-year period involved pregnant women who had hypertension before being seen initially in the first or early second trimester.[6] Once they had learned blood pressure self-monitoring, half of the women who had taken medications (usually diuretics) before pregnancy were able to discontinue these medications because of consistently normal blood pressure values outside the clinic. Conversely, methyldopa (Aldomet) alone or in combination with hydralazine or a β-blocking drug was started in those patients whose blood pressures were elevated both at home and in the clinic.

Performing a large randomized study to test the value of blood pressure self-monitoring during pregnancy is extremely difficult because of ethical considerations and the limited number of study cases. We have used for comparison a historical control group of chronically hypertensive women monitored only at clinic visits.[6] The same written protocols were followed for both control and self-monitoring groups. The control group showed a twofold increase in the number of patients for whom antihypertensive medications were prescribed by the end of pregnancy, in contrast to a slight decrease in the number needing medication in the self-monitoring group.

Our findings support those by others that antihypertensive medications for women with mild chronic hypertension are useful in diminishing maternal complications but not necessarily improving perinatal outcomes.[6] When elevated blood pressures persist at home, the patient's concerns are warranted, and we have recommended a reduction of activity, beginning or altering drug therapy, or admission to the hospital for closer observation. We continue to support the concept that chronically hypertensive women may be managed at home if blood pressures are borderline or mildly elevated in the clinic but normal at home. Nonpharmacologic actions (mainly increased bed rest) may contribute to feelings of well-being, which in turn lower blood pressure levels.

Blood pressure self-monitoring should lower the cost of obstetric prenatal care. The medical supplies needed are a blood pressure kit and printed recording sheet. Fewer antenatal hospitalizations and more precise drug prescribing of fewer drugs have been documented. We previously found that antepartum hospitalization for hypertension-related concerns was more than twice as common for mothers whose blood pressures were monitored only in the clinic or office than for those with follow-up both at home and in the office.[6]

Blood pressure devices

Portable electronic and mechanical aneroid devices suitable for home use may be purchased at most retail department and pharmacy stores, and their reliability has been described.[7] Approximately one third of these kits are simple mechanical devices that require reading a pressure gauge. These are called mechanical aneroid ("without liquid") models to distinguish them from those using a column of mercury. Most mechanical monitors are designed for one-handed use. Many have a stethoscope bell attached to the cuff for easy positioning over the brachial artery. With some, the patient must wedge the bell under the cuff, and most cuffs are marked to help position the bell correctly.

The remaining models are battery-operated portable electronic units that display pressures digitally. These oscillometric devices sense blood flow sounds as the cuff is deflated automatically. Cuffs on less expensive models are semiautomatic and must be pumped manually. Proper cuff placement is important. These units are

more sensitive to arm movements and may give a false reading or "error" message unless the patient remains still.

The electronic devices are virtually foolproof. We have found them to be easier for our pregnant patients to use than mechanical aneroid types.[8, 9] They do not require a stethoscope, and the digital displays are easy to read. All the cuffs close with Velcro fasteners, and most are easy to place on one's own arm. Each instrument has an air pump with a slow deflation rate (2 mm/sec) to improve sensitivity. Because of their greater complexity and sensitivity, they are more likely to become inaccurate and should be recalibrated at the doctor's office at least once yearly. One advantage is patient ease in recordings when in the lateral recumbent position.

We have found standard cuffs to fit most pregnant adults, but women with large upper arms (≥35 cm circumference) may have trouble.[10] Using a standard cuff (12 × 23 cm) that is too small may cause falsely elevated blood pressure readings. Larger cuffs (15 × 33 cm), available from medical supply houses, can be used with any mechanical aneroid model. Electronic models may require cuffs with special sensors, not always available in larger sizes.

Ratings in a *Consumer Reports* publication[7] showed that, as a group, the mechanical aneroid models were more accurate than the more complex electronic models. This finding may be explained by the electronic circuitry, which could not sense arterial signals quite as well as a person listening carefully with a stethoscope. Although they were found to be off by up to 5 to 7 mm Hg, the electronic units were rated as good enough for tracking long-term progress.[7-9]

With prices ranging from $18 to $30, many of the top-rated mechanical aneroid models would be good choices, depending on their features. They do require dexterity, good hearing, good eyesight, and some training and practice. We prefer that patients purchase the electronic models for ease of application and for minimizing interobserver bias.[8] The most compact electronic brands (Marshall, Pollenex, Norelco, Sunbeam) cost less than $40 and appear to be as accurate as others priced up to $100.[9]

Patient selection and instruction

Blood pressure self-monitoring is indicated for all pregnant patients with blood pressure of 140/90 mm Hg as measured in the clinic. This includes patients with chronic hypertension or elevated blood pressure first noted during the current pregnancy. Persistently elevated blood pressure places the patient at risk for needing antihypertensive medication, requiring preterm delivery, and having unfavorable perinatal outcome.

We think that with proper instruction and sufficient practice, patients or family members can become confident and aware of conditions related to changes in measured blood pressure. Our patients are taught how to measure and record blood pressure by a well-trained nurse. A dual-headed stethoscope teaches the patient the Korotkoff I, IV, and V sounds. The ones most accurate for pregnancy are Korotkoff I and IV since pregnancy is a high-cardiac-output condition.[11] The problem of whether to use the Korotkoff IV or V sound can be handled by recording all three (i.e., 140/90/86). We keep several blood pressure kits in our clinic for instruction and use by patients.

We have found that cuffs vary in ease of application; particularly simple are those with a slide bar on a mechanical aneroid unit.[8] Placement of the cuff and stethoscope (if necessary) by the patient should be observed closely. The cuff is to be positioned on the same arm each time at the level of the heart, with the sensor placed over the brachial artery.[12] The cuff is then insufflated to a value between 150 and 170 mm Hg. We prefer the left arm.

A stethoscope with two sets of ear pieces, one for the patient and one for the nurse, assures that the patient is reading the blood pressure correctly on a mechanical aneroid unit. The patient then records the blood pressure in a similar fashion each time. Diastolic pressures measured at Korotkoff IV sound correspond better to the intraarterial diastolic pressure. During pregnancy the difference between diastolic recordings at Korotkoff IV and V sounds can be remarkable, and sometimes Korotkoff V sound is 0.[13] The explanation is that the pregnant woman has a hyperkinetic circulation. It is recommended that all three sounds be recorded (i.e., I, IV, and V) to avoid confusion when others examine the patient.

Blood pressure is measured most easily with the patient in a sitting position. Readings using only the right arm as the patient reclines in a left lateral recumbent position may be inaccurate during pregnancy, since any measured decrease in blood pressure does not necessarily represent a fall in real blood pressure as measured in the ascending aorta.[14] We have demonstrated that blood pressures measured in the lateral recumbent position throughout pregnancy can be comparable; that is, the twelfth week of gestation can be compared to the twenty-fourth and thirty-sixth weeks since the enlarging uterus does not effect cardiovascular changes in the lateral position. There have been no prospective studies done that used both sitting and lateral recumbent positions in pregnancy.

Blood pressures have been reported to be lowest in the morning and highest in the afternoon, highest sitting or standing and lowest in the lateral recumbent position; hence patients are instructed to take their

daily measurements in the morning before rising and in the afternoon before resting. When position is taken into account, any decrease in blood pressure during the night in any individual is actually substantially smaller than the measurement suggests.[15]

The measurement of blood pressure by our patients and office staff requires the same methodologic rigor as other medical procedures. Failure to carry out these procedures markedly reduces their value in prenatal care and clinical research projects. It has been identified that a trained observer can reproduce blood pressure measurements ± 4 mm Hg, whereas "clinic values" may vary by 10 mm Hg. It is also interesting that "clinic values" showed more than 50% of readings ending in zero, whereas the trained observer showed only 14% ending in zero. Measurement devices must be standardized and regularly maintained.[11]

Patients are given a form on which to report their readings. These reports are placed on the prenatal chart at each office visit. Additionally, patients are asked to record any situation that may explain fluctuations in the blood pressure, such as unusual activities or interpersonal interactions or any occasion on which medications were not taken.

Criticisms and future needs

Limitations with self-administered blood pressure measurement must be considered along with benefits. The primary concern is inadequate training in measurement technique, leading to inaccurate measurement and misinterpretation of the measurements.[11] In-service training of all personnel utilizing a two-headed stethoscope will go a long way toward correcting errors and casualness of the readings. Normotensive values are reassuring, while elevated readings obtained through self-measurement are only an indication that further evaluation is needed. The diagnosis of hypertension can be made only by a physician who interprets the significance of the blood pressure readings along with other data relevant to the individual patient.

Blood pressure self-measurement may be more suitable for some patients than others. Although the situation is uncommon, some persons may become overly conscious of blood pressure and alarmed at fluctuations associated with daily living. Misinterpretation or overreaction to one or more measurements may cause distress without proper medical consultation. A careful education program on the use of the device and the meaning of the results is necessary. We have seen no deleterious effects on patients because of increased knowledge of daily blood pressure values. Many have said it allayed their apprehension to know the readings and to be part of the health care team.

Accuracy of the two types of devices (mechanical aneroid and electronic) has been tested in pregnant patients; both types provide a reasonable means of providing blood pressure self-measurement.[8, 9] We recommend that any device be compared with a standard mercury sphygmomanometer found in the clinic. The random-zero mercury sphygmomanometer, when used properly, is still the standard against which all other blood pressure measurement devices are compared.[3, 11] The greatest emphasis should be placed on the precision of the diastolic reading. Diastolic pressure changes as small as 5 mm Hg can shift the diagnosis from mild to moderate hypertension or from mild hypertension to normal.[1] The variation of taking the blood pressure may vary by 10 mm Hg. Nearly every monitor has given averaged readings quite close to the nurse's—typically within 4 mm Hg or less. As mentioned previously, measurements with a cuff that is too small may result in falsely elevated readings in an obese person.

Regardless of the type of equipment, patients benefit from written instructions on how to measure blood pressure. The manufacturer's printed instructions accompanying the devices usually specify that the arm be supported and bared and that an appropriate-sized cuff be used. The obstetrician who recommends self-measurement should provide or arrange for patient instruction in measurement, initial and periodic calibration, and record-keeping techniques.

Users of any device should also be provided with information about where to obtain follow-up information after a series of elevated readings. We counsel our patients to be aware that readings less than 140/90 mm Hg are considered normal, although a diastolic pressure of 85 to 89 mm Hg during pregnancy warrants further observation. A single elevated reading does not constitute a diagnosis of hypertension but is only a value. The patient should be instructed to contact the office promptly if readings remain elevated. Pregnant women known to have hypertension should continue their drug therapy and report blood pressure to the obstetrician at regularly scheduled visits.

Noninvasive ambulatory blood pressure monitoring devices have become available for the purpose of more carefully evaluating adults with preliminary clinical diagnosis of mild to moderate hypertension.[4] These electronic digital monitors record blood pressures continuously for 24-hour periods. Averaged readings are more informative to the physician and are thought to be more predictive of target organ involvement and cardiovascular complications than casual monitoring in the clinic.[4] Although its use in pregnancy has not yet been described, this means of monitoring may permit repeated measurement and recording of blood pressures over protracted time periods as subjects continue routine activities. However, high cost and questionable long-term accuracy substantially limit its routine clinical use.[3]

More prospective studies during pregnancy are needed to confirm our observations about the benefits of blood pressure self-monitoring, as well as the position in which the blood pressure should be taken (i.e., sitting or lateral recumbent). Clinical research during pregnancy using both office and home blood pressure determinations should also be more appropriate for evaluating the efficacy of antihypertensive therapy. Blood pressure self-monitoring should permit safer management of the majority of study pregnancies outside the hospital or earlier hospitalization before the next office visit if conditions worsen. We often encourage patients to check urine for protein by dipstick at home.

We consider blood pressure self-measurements to be complementary to monitoring techniques such as maternal capillary blood glucose determinations and uterine activity monitoring for active patient participation at home. A more sophisticated technique of measuring maternal blood pressure in the home with an automatic microcomputer-assisted instrument (Dinamap, Applied Medical Research Corporation, Tampa) with telemetric transfer of information to the clinic may be appealing in certain pregnant women with hypertension.[5] Such indirect determinations of blood pressure have been evaluated during pregnancy and found to eliminate the problem of interobservor error which otherwise becomes evident when blood pressure is checked antenatally.[16] This concept also requires further investigation, since it offers promise not only of reducing the cost of prenatal care but also of improving the pregnant woman's acceptance of the need for hospitalization for hypertension. This may be one method of picking up episodic hypertension during the day so that appropriate therapy or workup can be done.

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Dissection of the cardinal ligament in radical hysterectomy for cervical cancer with emphasis on the lateral ligament

Yoshihiko Yabuki, MD, Akihiro Asamoto, MD, Tsutomu Hoshiba, MD, Hideaki Nishimoto, MD, and Shuichi Kitamura, MD

Kanazawa, Japan

Surgical experience with carcinomas of the uterus and rectum has provided new insights into the surgical anatomy of a lamina, which separates the paravesical space from the pararectal space. It has been proved that each of the lamina consists of the cardinal and lateral ligaments and pelvic splanchnic nerves, descending in the following order. The cardinal and lateral ligaments, as a connective stalk, insert into the lateral walls of the uterus and rectum extending from the inner aspect of the pelvic wall. Clarification of this structural relationship led to the development of a new procedure for the dissection of the cardinal ligament in radical hysterectomy, while still preserving the lateral ligament. This facilitated systematic dissection of the cardinal and uterosacral ligaments with posterior manipulation, leading to a reduction in blood loss and to prevention of brisk bleeding from the venous plexuses. (AM J OBSTET GYNECOL 1991;164:7-14.)

Key words: Cardinal ligament; lateral ligament; radical hysterectomy; cervical cancer; ultrasonic surgical aspirator

Dissection of the cardinal ligament is one of the most critical procedures in radical hysterectomy, requiring skills of the highest degree. Over the years this procedure has been greatly influenced by individual surgeons and the differences in their techniques. The 5-year survival rate of patients who have surgical treatment for uterine cancer has already peaked[1] and to improve it there must be a narrowing of these differences and safer techniques. Today's dissection of the cardinal ligament owes much to the groundwork of Latzko (1919),[2] Okabayashi (1921),[3] and Meigs (1944)[4] who improved the original Wertheim operation.[5] They had a common factor in their surgical procedure, which was to expose the paravesical and pararectal spaces and then separate the retinaculum uteri into pars anterior, medial, and posterior, followed by resection of each pars individually. This made systematic and extensive resection of the parametrial ligament possible.

With regard to the cardinal ligament dissection, Meigs[6] improved the Wertheim operation in a comparatively orthodox manner by mere dissection of the Mackenrodt ligament, whereas Latzko[7] and Okabayashi[3, 8] pursued a radical approach by resection of the ligament, lateral stalk of the pelvis, deep down in the pelvic floor. This procedural difference made definition of the cardinal ligament unclear. Specifically, the anatomy and operative manipulation of the inferior border of the cardinal ligament was not always clearly defined. An operation performed with the boundaries of the base of the ligament and its adjacent organs undefined is in danger of damaging blood vessels and nerves by invasion into adjacent tissues. Especially, unskilled technique may cause an unexpected massive hemorrhage.

Kobayashi[9, 10] improved the Latzko and Okabayashi operation by separating the cardinal ligament into the pars nervosa and pars vasculosa after opening the paravesical and pararectal spaces completely to the superior surface of the levator ani so that the vesicular function was preserved by leaving the pars nervosa behind. This manipulation made more detailed explanation of the anatomy of the cardinal ligament very clear, but it was not stated why a part of it remained in the pelvirectal space.

With a view to further understanding the relationship between the parametrium and paraproctium, we performed operations for advanced rectal cancer and came to the following conclusions[11, 12]: (1) Metastasis of the cardinal lymph nodes found in many advanced rectal cancers suggested some continuity between the parametrium and paraproctium, and (2) After an anterior resection of the rectum together with a radical hysterectomy, a view of the dissected end of the ligament on the inner aspect of the pelvic wall is the same as that by radical hysterectomy with a simultaneous resection of the pars vasculosa and pars nervosa (i.e., the Oka-

From the Department of Obstetrics and Gynaecology, Ishikawa Prefectural Central Hospital.
Reprint requests: Yoshihiko Yabuki, MD, Department of Obstetrics and Gynaecology, Ishikawa Prefectural Central Hospital, Minami-Shinbo Nu 153, Kanazawa, Japan 920-02.
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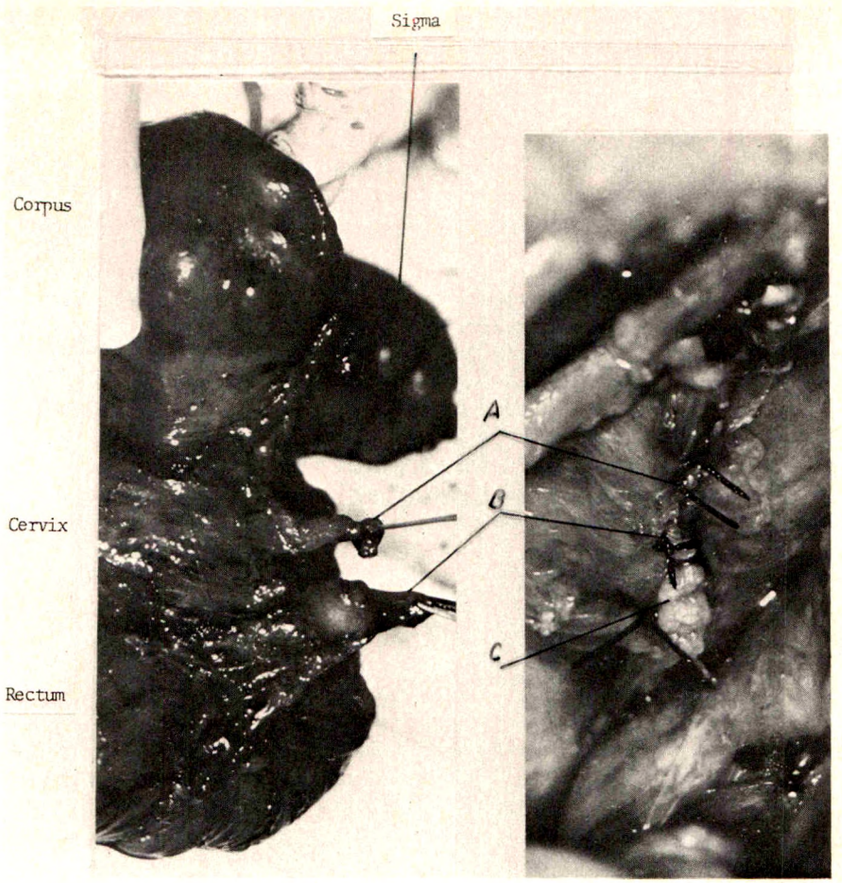


Fig. 1. *Left,* specimen removed by combination of rectal transection and radical hysterectomy for rectal cancer. *Right,* Severed ends on lateral wall of pelvis. Cardinal and lateral ligaments form laminae supporting uterus and rectum. *A,* Deep uterine vein and its severed end; *B,* lateral ligament and severed end of middle rectal artery and vein; *C,* connective tissue containing pelvic splanchnic nerves.

bayashi operation). This finding coincides with that of a deep pelvic dissection with total pelvic exenteration reported by Mattingly.[13]

Furthermore, when dissecting the cardinal ligament, the visceral branch of the internal iliac vessels (i.e., the deep uterine vein and middle rectal vessels) are exposed at their bifurcation and followed up to the runnings. The results led us to make the following inferences[12]: (1) In conventional radical hysterectomy,[3, 7, 9, 14, 15] the Mackenrodt ligament and lateral ligament,[16] which connects the rectum to the posterolateral pelvic wall, in which the middle rectal vessels pass at the level of the third sacral vertebra, have not been clearly differentiated, and (2) So-called cardinal ligament excised in many conventional radical hysterectomies may be a combined connective stalk consisting of the Mackenrodt and lateral ligaments and sometimes even the pelvic splanchnic nerves.

These inferences are necessary to clarify the anatomic relationship among the cardinal and lateral ligaments and pelvic splanchnic nerves. To our knowledge, no textbook of gynecosurgical procedures or anatomy is available that describes these two ligaments in a mutually related manner. Only the concept of Gefäß-Nerven-Leitplatte des Beckens as described by E. Pernkopf[17] (connective stalk, coated with visceral pelvic fascia, connects the lateral pelvic wall and the organs of the lesser pelvis) gives any suggestions similar to our opinions about the cardinal ligament.

In this article we describe our method of dissecting the cardinal ligament on the basis of the knowledge and findings of the surgical anatomy of the cardinal and lateral ligaments derived from operations for rectal cancer. It is hoped that our hypotheses will stimulate discussions among the readership on this matter and further verifications will be pursued.

Method

Subjects consisted of 41 patients with uterine cancer and 10 patients with rectal cancer on which we performed radical hysterectomy. In accordance with the Okabayashi-Meigs method, radical hysterectomy for uterine or rectal cancer was carried out by the following procedure: abdominal incision, severance of the round

and pelvic infundibular ligaments, open the peritonium to the iliac fossa, exposure of the paravesical and pararectal spaces, dissection of the intrapelvic lymph nodes, manipulation of the opposite side, separation and severance of the uterine artery, isolation of the ureter, separation and severance of the cardinal ligament, manipulation of the opposite side, separation of the rectum from the uterus, severance of the uterosacral and rectovaginal ligaments, manipulation of the opposite side, separation of the bladder, separation and severance of the superficial and deep layers of the vesicouterine ligament, resection of the paravaginal connective tissue, manipulation of the opposite side, transection of the vagina, and closure.

Separation and removal of the cadinal ligament and lymphatic tissues were carried out in part with an ultrasonic surgical aspirator (Cavitron Surgical Systems Inc., Stamford, Conn.). The operative condition of the ultrasonic surgical aspirator was as follows: an ultrasound frequency of 150 to 210 μm with a suction pressure of 200 to 400 mm Hg and an irrigation rate of 30 to 40 cc per minute. The Student t test was used to determine whether there was a significant difference in blood loss between patients who underwent radical hysterectomy before 1985 and those after 1987. The level of significance was set at 0.05 α-level. With consideration of the Japanese physique, we used the modified Broca index of weight/(height − 100) × 0.9 to express the degree of obesity.

Results

Surgicoanatomic relationship between the cardinal and lateral ligaments. Fig. 1 shows the specimen tissue for rectal cancer and its pelvic severed end. The operation was carried out by rectal transection and radical hysterectomy. Intraoperative observation and postoperative angiography of the surgical specimen showed that the vessels on the photomicrograph of the dissected ligament (Fig. 2) are in the following descending order: uterine artery, deep uterine vein, and middle rectal artery and vein. These findings indicate that the cardinal and lateral ligaments and pelvic splanchnic nerves form a lamina, as schematically shown in Fig. 3, which connects the uterus and rectum with the lateral inner aspect of the pelvic wall. The space caudal to the lamina is the paravesical space (actually the paravesical fossa and caudal part of the pararectal fossa), whereas the one that is on the cranial side is the pararectal space (actually the cranial part of the pararectal fossa).

All major blood vessels in the lamina are visceral branches of the internal iliac vessel in which the uterine artery and the superficial and deep uterine veins pass through the cardinal ligament, whereas the middle rectal artery and vein pass through the lateral ligament. As shown in Fig. 4, the deep uterine vein passes through the cardinal ligament at the level of the ischial

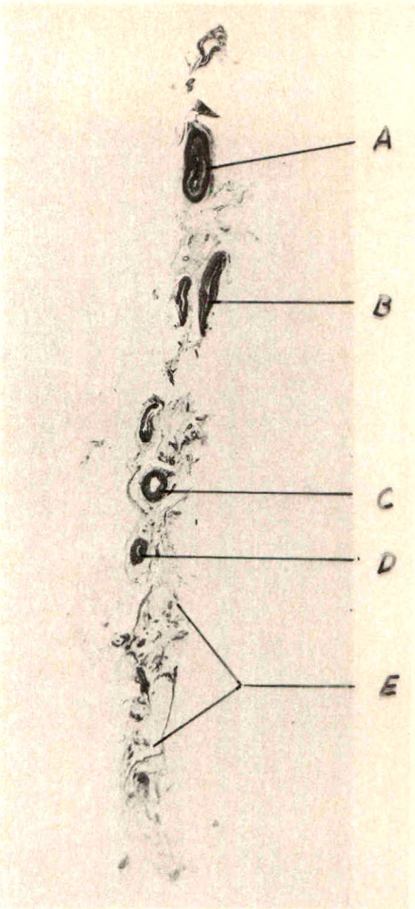


Fig. 2. Photomicrograph of resected laminae. (Elastica van Gieson stain; ×1.) *A*, Uterine artery; *B*, deep uterine vein; *C*, middle rectal artery; *D*, middle rectal vein; and *E*, pelvic splanchnic nerves.

spine and joins the internal iliac vein. The middle rectal artery branches mainly from the common trunk of the inferior gluteal and internal pudendal arteries and travels inferiorly along the deep uterine vein to the side of the rectum. The middle rectal vein passes through a similar course with a number of aberrations.

Dissection of the cardinal ligament. When the operation has proceeded as far as isolation of the ureter, the lamina becomes visible resembling a bridge spanning the paravesical and pararectal spaces. The dissection of the cardinal ligament commences with a resection of the strong fibrous tissue, that is, fascia pelvis visceralis as described by E. Pernkopf,[17] constituting the anterior and posterior surfaces of the lamina. This fibrous tissue is separated and severed accordingly. After removal of the fascia, the intraligamental lymph nodes and fibrous and fatty areolar tissues are removed with a pair of hookless dissecting forceps and an ultrasonic surgical aspirator or a surgical suction apparatus at a pressure of approximately 200 mm Hg, thereby re-

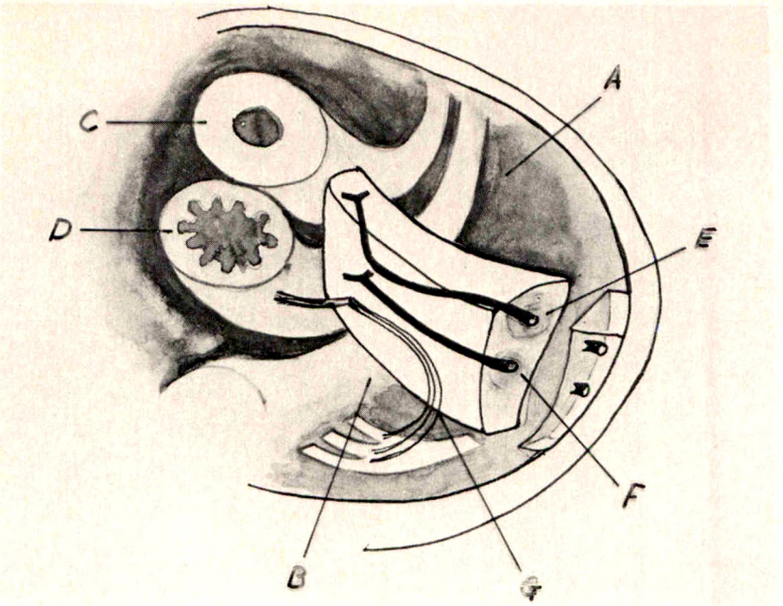


Fig. 3. Laminae connecting pelvic inside of wall with uterus and rectum (view from sacral side). *A*, Paravesical space; *B*, pararectal space; *C*, uterus; *D*, rectum; *E*, cardinal ligament containing uterine artery and vein; *F*, lateral ligament containing middle rectal artery and vein; and *G*, pelvic splanchnic nerves.

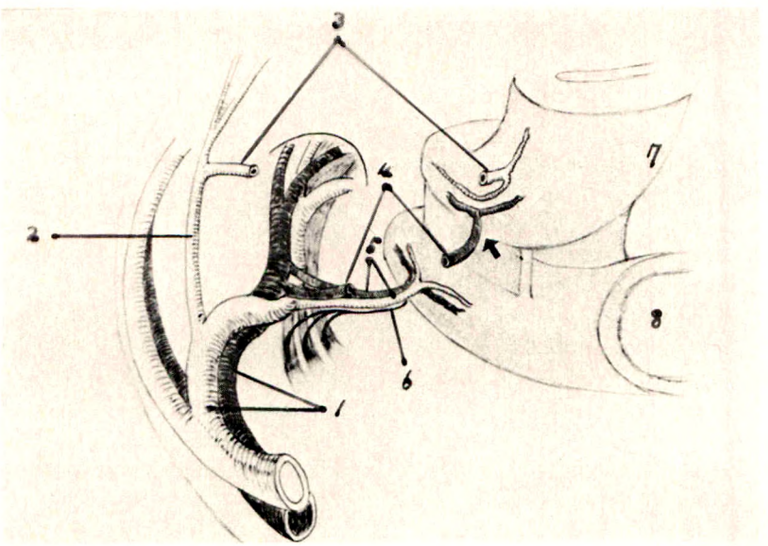


Fig. 4. Illustration indicates relationship between visceral branches of internal iliac vessel with uterus and rectum. Deep uterine vein does not join directly into uterine cervix, but forms stalk with middle rectal artery and vein. *1*, Internal iliac artery and vein; *2*, umbilical artery; *3*, uterine artery; *4*, deep uterine vein; *5*, middle rectal artery and vein; *6*, pelvic splanchnic nerves; *7*, uterus, and *8*, rectum.

vealing the deep uterine vein and the lateral ligament containing the middle rectal vessel and neural part below it (Fig. 5).

The exposed deep uterine vein and lateral ligament are separated from each other by insertion of Kelly's forceps. The former is ligated and severed on the pelvic side of the vessel's bifurcation (Fig. 6). The uterine side of the severed end of the deep uterine vein is raised,

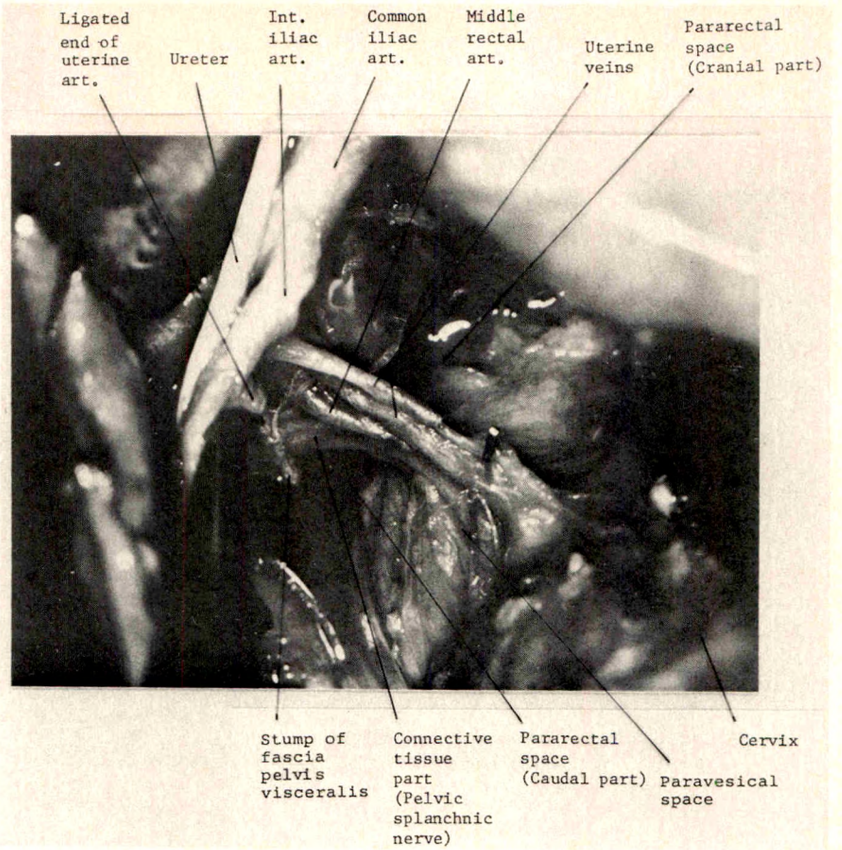


Fig. 5. Excision of fascia covering ligaments after developing paravesical space and pararectal space exposes vascular part and part of connective tissue. According to standard method for cardinal ligament dissection as used in Japan, ligaments are cut through a tunnel constructed between pars vasculosa and pars nervosa or beneath pars nervosa.

separating its distal segment from the lateral ligament up to the uterine cervix. In this way, both the cardinal and lateral ligaments are separated in their entirety and the rectovaginal ligament appears at the separated end (*arrow*, Fig. 4). At this time, care must be taken of hemorrhaging as a result of invasion into the rectouterine venous plexus. Finally, tissues surrounding the lateral ligament are adequately removed by the use of a ultrasonic surgical aspirator, completing the dissection of the cardinal ligament.

The tissue specimen (Fig. 7) removed by the conventional method is compared with that removed by the lateral ligament saving method. In the latter case the pelvic splanchnic nerves remain intact.

Benefits of the new surgical procedure. The amount of blood loss was measured at 30-minute intervals in 20 patients who underwent standard radical hysterectomy before 1985, at which time we realized the presence of the lateral ligament. Two years after the establishment of our new operative measure from 1987 through 1988 we did a comparison of blood loss on 21 patients with that of the prior group of 20 patients (Fig. 8). Table I shows operative information on patients of both groups. The time course of the operative procedure was judged from the anesthetic record, the intraoperative photographing time, and our operative speed experience. Blood loss during cardinal ligament dissection was significantly reduced in the latter group for the following reasons: (1) clarification of laminae anatomy by means of ultrasonic surgical aspirators; (2) avoidance of complete exposure of the pararectal space by disclosure of the superior surface of the levator ani only as required in the standard radical hysterectomy; and (3) systematization of posterior manipulations such as dissection of the uterosacral ligament (Fig. 9) because of the preservation of the lateral ligament. These improvements led to the prevention of intraligamental rupture of vessels and breakage of the venous plexuses

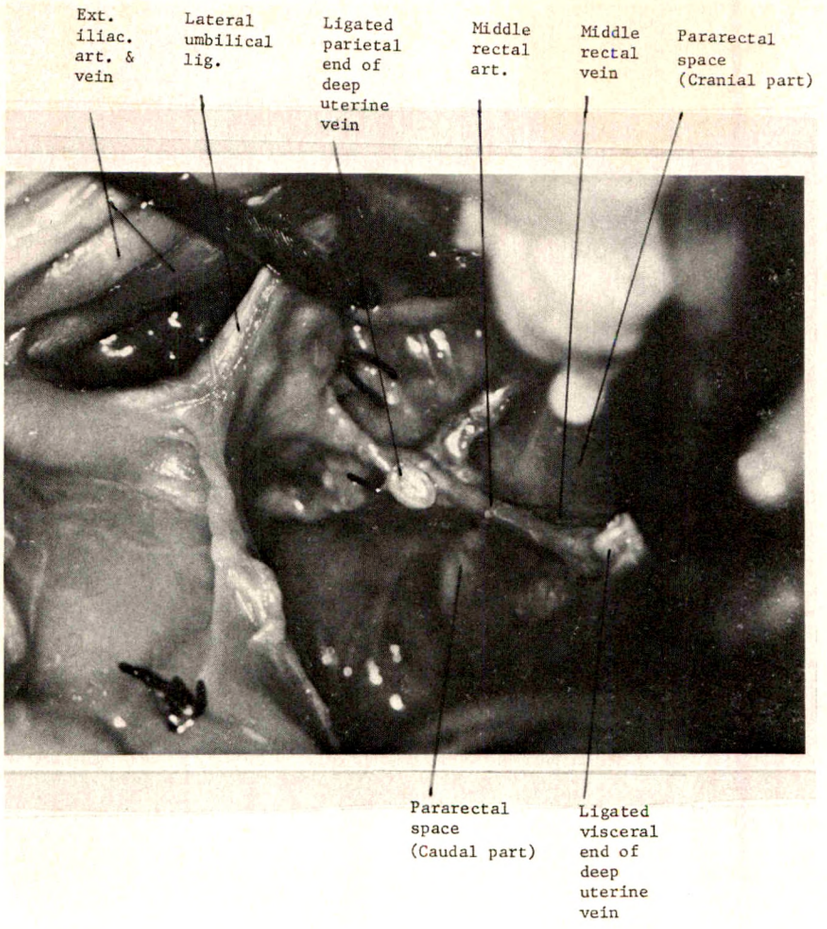


Fig. 6. Middle rectal artery and vein that become visible after severance of deep uterine vein.

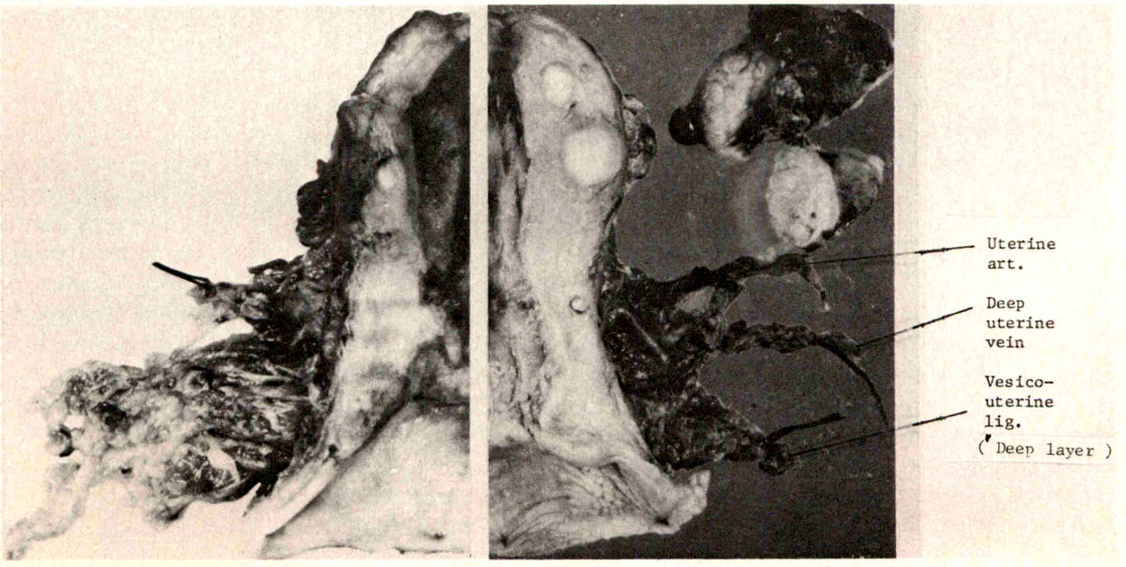


Fig. 7. *Left,* Specimen removed by conventional dissection of the cardinal ligament for cervical cancer. *Right,* Specimen removed by our lateral ligament-preserving method. Conventional technique leaves cut-end mass of ligament in pelvic wall, posing problems for curability.

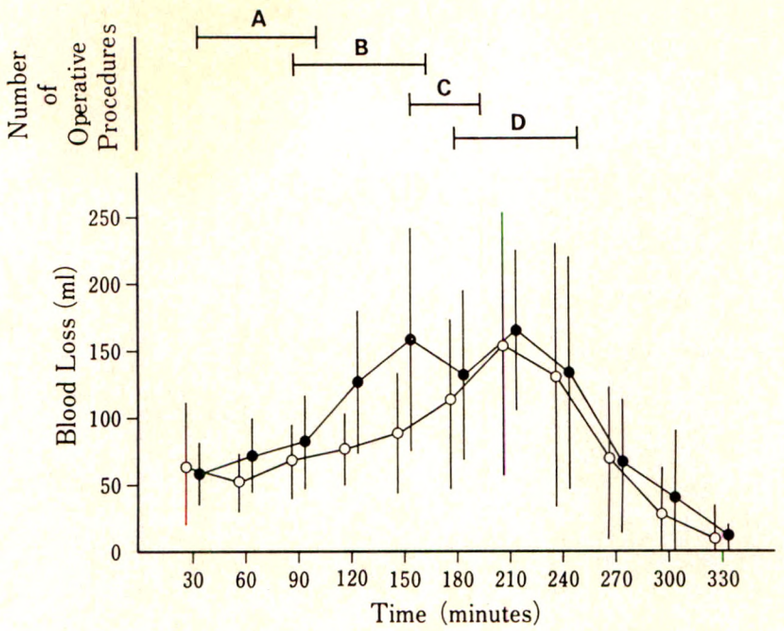


Fig. 8. Comparison of intraoperative bleeding measured at 30-minute intervals, between standard radical hysterectomy (total volume, 1092 ± 359 ml; operation time, 300 ± 32 minutes) and radical hysterectomy with our method (total volume, 850 ± 290 ml; operation time 296 ± 36 minutes). In the latter case blood losses measured at 120 and 150 minutes were 75 ± 27 and 86 ± 44 ml, respectively. These values are significantly smaller than counterparts of 127 ± 51 and 158 ± 83 ml for the former ($p < 0.05$). *A*, Dissection of intrapelvic lymph nodes; *B*, dissection of cardinal ligament; *C*, severance of uterosacral and rectovaginal ligaments; *D*, severance of vesicouterine ligament and paracolpium.

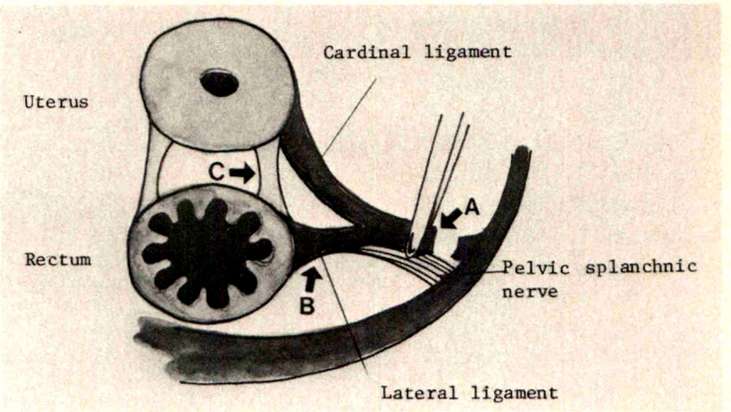


Fig. 9. When cardinal and lateral ligaments are clamped and severed together *(arrow A)*, complete separation between uterus and rectum necessitates severance of not only uterosacral ligament or rectovaginal ligament *(arrow C)* but also attachment of lateral ligament to rectum *(arrow B)*.

in the vicinity of the pelvic wall, rectum, and anterior surface of the sacrum.

Comment

The difference in the operative procedure of the cardinal ligament dissection among the Wertheim, Meigs, Latzko, and Okabayashi operations is that the former two adopt dissection of the Mackenrodt ligament, whereas the latter adopt deep dissection of the lateral pelvic stalk. The principle of surgical treatment for cancer necessitates extensive removal of affected tissue while maintaining functions of adjacent organs as much as possible. Although the preservation of the lateral ligament seems inconsistent with a radical cure, no progress will be possible without overcoming this dilemma.

Table II shows comparative 5-year survival rates for uterine cancer among the Wertheim and Okabayashi operations as cited from Kobayashi's work,[9] and the Meigs operation.[18] A comparison of the values for these

Table I. Information on patients who had surgery

	No. of patients	*Age (yr)*	*Clinical stage*		*Broca index*	*Intraoperative blood transfusion*	*Blood loss (ml)*
			I	*II*			
Standard method	20	53.4 ± 8.2	6	14	1.16 ± 0.16	955.8 ± 417.4	1029 ± 359
Our new method	21	52.6 ± 9.5	4	17	1.07 ± 0.19	777.8 ± 642.5	850 ± 290

Table II. Comparison of 5-year survival of patients operated on for cervical cancer between the Wertheim, Okabayashi, and Meigs operation

	Wertheim (%)	*Okabayashi (%)*	*Meigs (%)*
Stage I	64.0	90.9	80.7
Stage II	46.6	43.3	60.7

three operative methods does not always confirm the need for deep pelvic dissection advocated by Okabayashi. In terms of 5-year survival rates for the Wertheim and Okabayashi operations, there was a significant difference for stage I cancers by the Okabayashi operation, but no significant difference for stage II cancers. This was probably because of the dependence of the prognosis on the presence of metastasis in the pelvic lymph nodes irrespective of the operative methods. Our experience further indicated that the Laztko and Okabayashi operations leave the massive severed end of the ligament in the inner aspect of the pelvic wall, posing a problem for recurrence and consequently limiting both operative procedures.

Whereas our operation followed the traditional procedure of the Okabayashi operation, it is characterized by its concise anatomy of the depth of the laminae (so-called cardinal ligament) and by a complete extirpation of the cardinal ligament (the Mackenrodt ligament) from the pelvic wall after exposure and resection of the cardinal blood vessels at the bifurcation of the trunk. In this case, an ultrasonic surgical aspirator is highly contributive. However, when an ultrasonic surgical aspirator is used, complete irrigation and suction throughout the operative field are imperative to eliminate the risk of disseminating viable malignant cells.[19] Furthermore, careful attention should be paid to prevent nerve injury as a result of the ultrasound and heated tip of the ultrasonic surgical aspirator.

In conclusion, our anatomic opinions on the cardinal ligament will also be of interest to surgeons who carry out operations for advanced rectal cancer or gynecologists who perform uterine transplantation in addition to operations for uterine cancer.

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Overexpression of HER-2/*neu* in endometrial cancer is associated with advanced stage disease

Andrew Berchuck, MD,[a, f] Gus Rodriguez, MD,[a] Robert B. Kinney, MD,[b] John T. Soper, MD,[a] Richard K. Dodge, MS,[c] Daniel L. Clarke-Pearson, MD,[a] and Robert C. Bast, Jr., MD[d, e]

Durham, North Carolina

Prior studies have shown that overexpression of HER-2/*neu* occurs in one third of breast and ovarian cancers and that overexpression is associated with poor prognosis. We used a monoclonal antibody to assess immunohistochemically the level of HER-2/*neu* expression in normal and malignant endometrium. In 24 normal endometrial samples light to moderate (1+ to 2+) staining for HER-2/*neu* was seen in the glands, and there was no variation in intensity of staining during the menstrual cycle. Among 95 endometrial adenocarcinomas, nine (9%) were found to have heavier staining for HER-2/*neu* than was seen in normal endometrium (3+). High expression of HER-2/*neu* was found in 27% of patients with metastatic disease compared with 4% of patients with disease confined to the uterus ($p < 0.005$). High HER-2/*neu* expression also was associated with absence of estrogen receptor ($p < 0.005$) and with increased mortality from cancer. Further studies are needed to determine the significance of HER-2/*neu* overexpression in endometrial cancer. (Am J Obstet Gynecol 1991;164:15-21.)

Key words: Endometrial cancer, HER-2/*neu* oncogene, endometrium

In the past decade the discovery of oncogenes has led to an increased understanding of the biomolecular abnormalities associated with malignant transformation. In this regard it has been shown that viral oncogenes are derived from cellular genes that normally participate in processes of cellular growth and differentiation. In transforming retroviruses and human malignancies these genes are thought to have acquired the ability to elicit malignant transformation by various mechanisms involving either mutation or overexpression. Several of the retroviral oncogenes that have been sequenced have been shown to be homologous with peptide growth factors or their cell membrane receptors. For example, v-*sis* encodes one chain of platelet-derived growth factor whereas v-*erb* B is derived from the gene encoding the epidermal growth factor receptor (c-*erb* B).

In contrast to retroviral oncogenes, the *neu* gene was first identified as the oncogene associated with the development of neuroblastomas in rats exposed to ethyl nitrosourea in utero.[1] In this animal model it has been shown that *neu* is oncogenic because of a single point mutation.[2] Because of the high degree of homology between *neu* and the epidermal growth factor receptor, it is thought that *neu* also encodes a growth factor receptor, although no ligand for *neu* has yet been identified.[3] Although the role of *neu* as a growth factor receptor remains speculative, overexpression of the human *neu* gene, known as HER-2/*neu*, or c-*erb*B2, has been reported to occur in approximately 15% to 40% of breast and ovarian cancers.[4-6] Slamon et al.[4, 5] have shown that overexpression of HER-2/*neu* usually is associated with HER-2/*neu* gene amplification (10% to 30%), whereas approximately 10% of breast and ovarian cancers overexpress HER-2/*neu* in association with a normal gene copy number. In addition, Slamon et al. found that HER-2/*neu* overexpression was associated with aggressive biologic behavior and poor survival in breast and ovarian cancer. A study performed by our group confirmed the association of HER-2/*neu* over-

From the Departments of Obstetrics and Gynecology (Division of Gynecologic Oncology),[a] Pathology,[b] Biostatistics,[c] Medicine,[d] Microbiology/Immunology,[e] and the Duke Comprehensive Cancer Center,[f] Duke University.

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Reprint requests: Andrew Berchuck, MD, Duke University Medical Center, Box 3079, Durham, NC 27710.

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expression with poor survival in advanced ovarian cancer and demonstrated that this effect is independent of residual tumor volume or histologic grade.[6]

This study is the first to examine HER-2/*neu* expression in normal endometrium and endometrial adenocarcinoma. We used a monoclonal antibody specifically reactive with the external domain of HER-2/*neu* to localize HER-2/*neu* immunohistochemically in frozen tissue sections. The level of HER-2/*neu* expression in endometrial adenocarcinomas was compared with that seen in normal endometrium. In addition, we examined the relationship between the level of expression of HER-2/*neu* and clinicopathologic features of endometrial cancers.

Material and methods

Patients. All 95 patients in this study with endometrial cancer underwent primary surgical therapy at Duke University between 1985 and 1990. Hysterectomy, bilateral salpingo-oophorectomy, and pelvic washings were performed in all cases. In most cases a sample of tumor was submitted for determination of estrogen and progesterone receptor levels. Steroid receptor analysis was performed as described previously with biochemical techniques.[7] Tumors with >7.5 fmol of specific binding per milligram of tissue protein were considered receptor positive. Pelvic and periaortic lymph node sampling was performed as deemed appropriate by the surgeon. In 36 cases lymphadenectomy was not performed for one of the following reasons: presence of extrauterine disease, poor medical condition of the patient, or cancer that was well to moderately differentiated and only minimally invasive. All histologic material from each case was reviewed by a single pathologist, and the histologic type, histologic grade, depth of myometrial invasion, and stage of each cancer (International Federation of Gynecology and Obstetrics) was determined. In addition, a sample of normal endometrium was obtained from the fundal area in 24 women who were undergoing hysterectomy for treatment of benign gynecologic disease. Designations of endometrial dating were assessed by the histologic criteria of Noyes et al.[8]

Monoclonal antibodies. TA-1 is a purified murine monoclonal antibody that was generated by immunization of mice with cells expressing the human HER-2/*neu* molecule[9] (Applied bioTechnology Inc., Cambridge, Mass.). This antibody binds to the extracellular domain of HER-2/*neu* and is not cross reactive with the epidermal growth factor receptor. We found that maximal staining of HER-2/*neu* was achieved with the use of TA-1 at a concentration of 0.5 μg/ml. This concentration of antibody was used to stain all the tissue samples in this study. We used purified mouse immunoglobulin G that is specific for nonhuman tissue (Coulter Immunology, Hialeah, Fla.) at a dilution of 1:100 as a negative control. Antikeratin AE1/AE3 (Boehringer Mannheim Biochemicals, Indianapolis) was used as a positive control.

Immunohistochemistry. All tissue samples were frozen in tissue Tek O.C.T. compound (Ames Division, Miles Laboratories, Elkhart, Ind.) within 30 minutes and stored at −70° C until analyzed. Then 6-μm-thick cryosections were mounted on gelatin-coated slides. The slides were air dried overnight at room temperature to enhance cellular morphologic features. The slides were fixed in acetone for 10 minutes at room temperature and air dried again for 30 minutes. Slides were then washed in phosphate-buffered saline solution three times for 5 minutes. Immunohistochemical staining was performed with Elite Vectastain ABC kit reagents (Vector Laboratories, Burlingame, Calif.) as described previously.[6] For this study staining was performed with the Shandon Cadenza automated immunostainer (Shandon Inc., Pittsburgh). The slides were developed for 4 minutes with the enzyme substrate diaminobenzidine (0.5% diaminobenzidine in 0.5% Tris buffer and 0.6% hydrogen peroxide). The slides then were rinsed for 10 minutes in running tap water, counterstained with 1% methyl green (Sigma Chemical Co., St. Louis), dehydrated, and mounted.

Immunohistochemical localization of HER-2/*neu* was evaluated by means of serial sections. First, a section stained with hematoxylin and eosin was reviewed to evaluate the histologic features. A negative control slide stained with nonspecific mouse IgG was examined to assess nonspecific staining. Next, a positive control slide stained with antikeratin antibody was examined to confirm the presence of viable epithelial or tumor cells in the section. In cases in which the negative and positive controls were not adequate, evaluations were repeated. Finally, the slide in which TA-1 was used as the primary antibody was examined. The intensity of staining was evaluated by two observers using a double-headed microscope. The identify of the patients was not known to the observers while the slides were being scored. The intensity was graded as follows: 0 (staining not greater than negative control), 1+ (light staining), 2+ (moderate staining), or 3+ (heavy staining). Differences between observers were resolved by consensus.

Statistics. Statistical analysis of data in this study was performed with Fisher's two-tailed exact test.[10]

Results

Expression of HER-2/*neu* was examined in normal endometrium from 24 patients (8 proliferative, 11 secretory, 5 postmenopausal). In all cases staining for HER-2/*neu* was confined to endometrial glands and was homogenous (Fig. 1). The intensity of staining in glands ranged from light to moderate (1+ to 2+), but

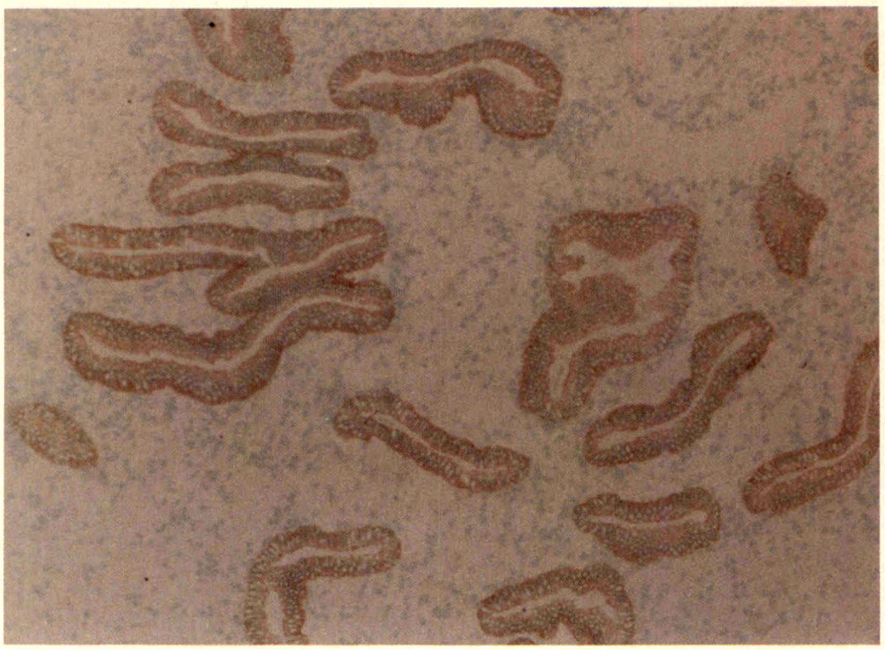


Fig. 1. HER-2/*neu* expression in normal secretory endometrium. Moderate (2+) staining for HER-2/*neu* is seen in endometrial glands.

there was no correlation between menstrual phase and the intensity of staining for HER-2/*neu*. Staining for HER-2/*neu* was not seen in endometrial stroma or myometrium.

The distribution of patients with endometrial cancer by FIGO stage is shown in Table I. Among these 95 patients, 73 (77%) had disease that was confined to the uterus. This included 7 patients with positive peritoneal cytologic results (stage IIIA). Twenty-two patients (23%) had extrauterine disease. In this study we observed the expected relationships between known prognostic factors. For example, increasing histologic grade was associated with a higher incidence of invasion of the outer one third of the uterine wall ($p < 0.05$). Invasion of the outer one third was seen in 7% of patients with well-differentiated cancer, 26% of patients with moderately differentiated cancer, and 43% of patients with poorly differentiated cancer. In addition, increasing histologic grade was associated with a higher incidence of extrauterine metastases ($p < 0.05$). Extrauterine metastases was seen in 7% of patients with well-differentiated cancer, 24% of patients with moderately differentiated cancer, and 43% of patients with poorly differentiated cancer.

Staining for HER-2/*neu* was observed in all 95 endometrial cancers. As in normal endometrium, expression of HER-2/*neu* in cancer was confined to the glandular component. Staining was not observed in the endometrial stroma or myometrium. In the cancer cells staining was strongest on the cell membrane, but cytoplasmic staining also was seen. In a given specimen, the intensity of staining of cancer cells was relatively homogeneous. Light to moderate (1+ to 2+) staining for HER-2/*neu*, similar to normal endometrium, was seen in 86 cases (91%) (Fig. 2, *A*) whereas heavy (3+) staining was seen in 9 cases (9%) (Fig. 2, *B*). There was a significant relationship between heavy staining for HER-2/*neu* and the presence of metastatic disease ($p < 0.005$). Among the 73 patients with disease confined to the uterus (including 7 with positive peritoneal cytologic results), 3 patients (4%) had heavy staining for HER-2/*neu*. In contrast, 6 of 22 patients (27%) with extrauterine metastases had heavy staining for HER-2/*neu*. Among the 22 patients with metastatic disease, heavy staining for HER-2/*neu* was seen more frequently in patients with intraperitoneal metastases (42%) than in those patients in whom metastasic disease was confined to lymph nodes (10%). In two cases in which peritoneal metastases were present, HER-2/*neu* expression was evaluated in both the primary tumor and one metastatic site. In both cases the intensity of staining was the same when the two sites were compared. This included one case in which normal (1+) HER-2/*neu* expression was seen and one case in which high (3+) HER-2/*neu* expression was seen.

Among the 95 cancers, 71 (74%) were histologically endometrioid, 12 (13%) were adenosquamous, and 12 (13%) were papillary serous. High (3+) HER-2/*neu* expression was noted in 8% of endometrioid cancers (6 cases) and 25% of papillary serous cancers (3 cases)

Table I. Relationship between FIGO stage and HER-2/neu expression in endometrial cancer

FIGO stage	*Total No. of patients*	*High HER-2/neu expression*	
		No. of patients	*%*
IA. Limited to endometrium	12	1	8
IB. Invades inner half of myometrium	38	2	5
IC. Invades outer half of myometrium	12	0	0
IIA. Endocervical glands involved	1	0	0
IIB. Cervical stroma involved	3	0	0
IIIA. Positive cytologic results	7	0	0
IIIB. Vaginal metastases	0	—	—
IIIC. Lymph node metastases	10	1	10
IVA. Bowel or bladder invasion	0	—	—
IVB. Distant metastases	12	5	42

(p = 0.08). None of the 12 patients with adenosquamous cancers had high levels of HER-2/*neu* expression.

The relationship between a high level of HER-2/*neu* expression and other known prognostic factors is shown in Table II. Although increasing histologic grade and depth of myometrial invasion were associated with a higher incidence of high HER-2/*neu* expression, these relationships were not statistically significant. For example, only 2 of 51 patients (4%) with cancer confined to the endometrium or invading the inner one third of the myometrium had high HER-2/*neu* expression compared with 7 of 44 patients (16%) with deeper invasion (p = 0.08). With respect to steroid receptor status, there was a significant relationship between HER-2/*neu* expression and estrogen receptor status (p <0.005). All 8 cancers with high levels of HER-2/*neu* expression in which steroid receptor levels were determined were estrogen receptor negative (Table II). With respect to progesterone receptor status, 4 of 8 cancers with high HER-2/*neu* expression in which progesterone receptor levels were determined were progesterone receptor positive.

In this study we found that high HER-2/*neu* expression was associated with an increased incidence of death from persistent or recurrent cancer (Table III). Overall, among the 86 patients whose cancers had light to moderate staining for HER-2/*neu*, 10% are dead of disease, compared with 56% of the 9 patients whose cancers had heavy staining. Among the three patients with stage I disease who had high HER-2/*neu* expression, one has developed recurrent disease. If only patients who presented with metastatic disease are considered, 5 of 16 patients (31%) whose tumors had light to moderate staining for HER-2/*neu* are dead of disease while all 6 patients whose tumors had heavy staining for HER-2/*neu* are dead of disease. Since most of the patients in this study underwent operation during the past 2 years, survival data are not mature enough to present as survival curves.

Comment

Although HER-2/*neu* is structurally similar to the epidermal growth factor receptor, we found that the distribution of these two cell surface molecules in the normal human uterus differs significantly. Previously, we and others have shown that epidermal growth factor receptor is expressed by endometrial glands, endometrial stromal cells, and myometrium.[13, 14] In contrast, in this study we found that expression of HER-2/*neu* in the uterus is confined to endometrial glands. Similarly, immunohistochemical studies of *neu* expression in the rat also have shown that *neu* is expressed primarily by epithelial cells.[13] Like epidermal growth factor receptor, however, we saw no cyclic variation in the intensity of staining for HER-2/*neu* during the menstrual cycle. Further studies are needed to clarify the physiologic significance of the differences in distribution of epidermal growth factor receptor and HER-2/*neu* in the uterus and to determine the role of these receptors in processes of normal growth and development in this organ.

Prior studies of HER-2/*neu* in human cancers have shown that overexpression of structurally normal HER-2/*neu* protein occurs in approximately one third of breast and ovarian adenocarcinomas.[4-6] This is the first study to examine HER-2/*neu* expression in a significant number of endometrial cancers. We found that 9% of endometrial adenocarcinomas had heavier staining for HER-2/*neu* than was observed in normal endometrial glands. Although high HER-2/*neu* expression was uncommon (4%) among early stage cancers, 27% of cancers that had metastasized were found to have have HER-2/*neu* expression. Interestingly, the highest incidence of HER-2/*neu* overexpression (42%) was found in patients whose tumors had spread intraperitoneally (stage IVB), similar to ovarian cancer. As in breast[4, 5] and ovarian cancer,[5, 6] HER-2/*neu* overexpression was found to be associated with poor prognosis in endo-

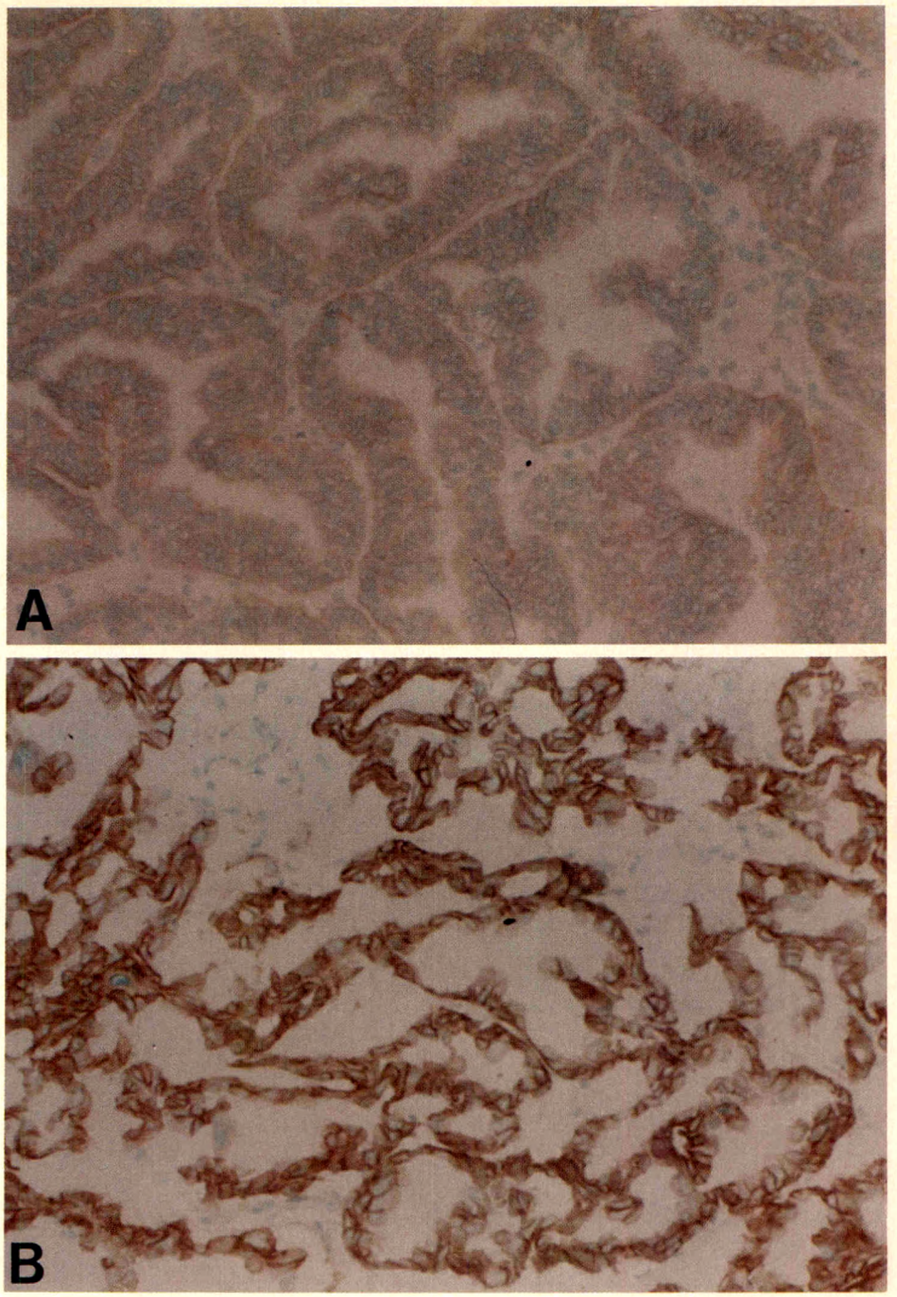


Fig. 2. HER-2/*neu* expression in endometrial cancer. **A**, Well-differentiated endometrial cancer with light (1+) staining for HER-2/*neu*. **B**, Moderately differentiated endometrial cancer with heavy (3+) staining for HER-2/*neu*.

metrial cancer. Among the 9 patients with HER-2/*neu* overexpression, one of three with disease that initially was confined to the uterus has developed recurrent disease, and all 6 patients who presented with metastases are dead of disease.

In breast cancer several studies have shown that expression of epidermal growth factor receptor and estrogen receptor is inversely related and that epidermal growth factor receptor expression is associated with poor prognosis.[14, 15] However, no relationship between HER-2/*neu* overexpression and estrogen receptor status has been noted in breast cancer.[16] Although endo-

Table II. Relationship between HER-2/neu expression and prognostic factors in endometrial adenocarcinoma

Prognostic factor	*Total no. of patients*	*High HER-2/neu expression*	
		No. of patients	*%*
Histologic grade			
Well-differentiated	28	2	7
Moderately differentiated	46	4	9
Poorly differentiated	21	3	14
Myometrial invasion			
None	12	1	8
Inner third	39	1	3
Middle third	21	4	19
Outer third	23	3	13
Extrauterine disease			
Absent	86	3	49
Present	9	6	27
Estrogen receptor			
Positive	40	0	0
Negative	41	8	20
Progesterone receptor			
Positive	46	4	9
Negative	33	4	12

Table III. Relationship between HER-2/neu expression and survival

Status	*HER-2/neu expression*			
	Normal (1+-2+)		*High (3+)*	
	No. of patients	*%*	*No. of patients*	*%*
No evidence of disease	72	84	2	22
Dead of intercurrent disease	1	1	0	0
Alive with disease	4	5	2	22
Dead of disease	9	10	5	56

metrial cancers, like breast cancers, frequently express estrogen receptor and may be responsive to endocrine therapy, in an earlier study of endometrial cancer we did not find a relationship between expression of epidermal growth factor receptor and estrogen receptor. In the present study, however, we did find a strong inverse relationship between high HER-2/*neu* expression and estrogen receptor status. In all cases in which high HER-2/*neu* expression was seen, estrogen receptor was absent. These results suggest that although both breast and endometrial cancers frequently maintain expression of steroid receptors, they may differ with respect to which oncogenes are involved in their pathogenesis. It is possible that increased HER-2/*neu* expression in estrogen-receptor-negative endometrial cancers reflects escape from normal steroid hormone–related growth regulatory pathways.

HER-2/*neu* overexpression appears to be an important prognostic factor in female reproductive cancers (breast, ovary, endometrium). If assessment of HER-2/*neu* expression is to be used in the clinical management of patients, however, a practical and reliable method must be available. In this and other studies,[6] we have used immunohistochemical techniques to compare the intensity of staining for HER-2/*neu* in frozen sections of cancers with that seen in frozen sections of the normal organ from which the cancer arose. The advantage of this method is that it allows direct assessment of HER-2/*neu* expression at a cellular level. Other methods in which tissue homogenates are used to measure levels of deoxyribonucleic acid, ribonucleic acid, or protein all are subject to error because of differences in the proportion of cancer cells relative to stromal elements in tumor samples. On the other hand, although results obtained with immunohistochemical techniques have been shown to correlate well with the Southern, Northern, and Western techniques,[5] immunohistochemical assessment has been only semiquantitative in the past.

Recently, however, a computerized image analysis program has been developed that allows quantitative assessment of immunohistochemical staining for HER-2/*neu*.[17] Cell lines with both normal and high expression of HER-2/*neu* are used as calibration standards so that day-to-day variation in staining intensity can be accounted for. The intensity of staining of cancer specimens is interpreted relative to that of the cell lines with known normal or high HER-2/*neu* expression. Since the antigenicity of HER-2/*neu* can be altered by paraffin embedding, however, it has been suggested that frozen tissue samples are most suitable for immunohistochemical evaluation. Presently, we are conducting a prospective study of the prognostic value of HER-2/*neu* in gynecologic cancers with the use of quantitative image analysis of immunohistochemical staining for HER-2/*neu* in frozen sections.

The precise significance of the relationship between HER-2/*neu* overexpression and the biologic behavior of female reproductive cancers (breast, ovary, endometrium) remains unknown. Several groups of investigators now are studying the role of HER-2/*neu* in neoplasia to determine if HER-2/*neu* overexpression is directly involved in, rather than merely associated with, the biomolecular events involved in evolution of cells with metastatic potential. If HER-2/*neu* overexpression is found to be a causal event in the process of tumor progression, this would suggest that the favorable prognosis of early stage endometrial cancer may be due, in part, to favorable tumor biologic characteristics, as reflected by the low incidence of HER-2/*neu* overexpression, rather than merely to early clinical detection. Conversely, ovarian cancers, which frequently overexpress

HER-2/*neu*, may be highly lethal not only because of their insidious clinical presentation but also because of more aggressive biologic behavior, as reflected by a high incidence of HER-2/*neu* overexpression.

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Early neonatal mortality and cesarean delivery in Mexico City

Jose L. Bobadilla, MD, PhD, and Godfrey J.A. Walker, MD, MFCM
Mexico City, Mexico, and London, England

The relationship between cesarean delivery and neonatal mortality is presented with information from 292 early neonatal deaths (cases) and 3098 survivors (controls) born in 25 hospitals in Mexico City during the summer of 1984. The overall rate of cesarean delivery was 27%. Variations between health agencies and different social groups were not related to obstetric risk, suggesting that a sizable proportion of the operations were probably unjustified. Babies of normal birth weight (≥2500 gm) delivered by cesarean section were 2.5 times more likely to die in the early neonatal period compared with vaginally delivered babies of the same weight. The excess of mortality could not be explained by the effect of maternal characteristics or complications or by differences in birth weight or gestational age. It is suggested that the conditions under which the operation was performed probably explain the increased risk of early neonatal death. It is likely that poor quality of resuscitation and respiratory care are implicated in the link between "unnecessary" cesarean section and early neonatal mortality. (AM J OBSTET GYNECOL 1991;164:22-8.)

Key words: Cesarean delivery, early neonatal mortality, Mexico City

Cesarean delivery rates have increased steadily in many countries over the past two decades.[1-7] Very high rates have been reported from Latin America ranging from 20% in public hospitals in Mexico City[8] to 27% in southern Brazil[9] and 49% in Campinas, Brazil.[2]

The reasons for the rise in cesarean section rates are complex and likely to vary between different places.[10, 11] Two major factors that influenced obstetricians are improvements in the safety of the procedure in terms of reduced maternal morbidity and mortality[12] and claims that increased rates of cesarean section have led to a decline in perinatal mortality.[13, 14]

Other factors that contributed to the rise in cesarean delivery include the availability of more precise technology to diagnose fetal distress,[15] the snowball effect produced by the policy of "once a section, always a section,"[16] higher rates of induction of labor,[17] higher proportions of women delivered of infants in large hospitals and of third-party payments for deliveries,[12, 18] and the practice of defensive obstetrics.[3, 19] The wide variation in rates of cesarean section between and within countries raises questions as to the effectiveness of the procedure in improving the prospects of early neonatal survival.[5, 20, 21]

Even in countries with adequate resources and good quality medical care, early neonatal mortality rates among cesarean deliveries are seldom reported separately and are often excluded from discussions on the benefits and risks of cesarean section.[22]

This study examines the relationship of the outcome of obstetric care in terms of early neonatal mortality (controlled for maternal risk status) to the adequacy of care, specifically cesarean delivery, provided at hospitals in Mexico City.

In the metropolitan area of Mexico City there are annually over 310,000 births of which about 85% occur in hospitals (unpublished data). Hospital care is provided through three systems: (1) the public assistance system of the Ministry of Health and Federal District Health Services; (2) the Social Security system of which the Mexican Institute of Social Security and the Institute of Social Security and Services for Employees of the Federal Government are by far the largest; and (3) independent private practice. The various systems are known to cater to different communities. In general, the poorest persons use the public assistance, manual and public workers use Social Security, and the more affluent use the private practice option.

Material and methods

Representative maternity units in hospitals of the three medical care systems were included in the study. Seven public assistance hospitals (covering an estimated 74% of births in this system), 14 Social Security hospitals (seven from each of the two major agencies and covering about one third of the births in this system), and the four private hospitals that reported over 2000 deliveries per annum were selected.

Over a 14-week period during the summer of 1984 information was collected on a sample of women delivered of infants in the study hospitals, the babies who survived to the seventh day after delivery, and all

From the Centre for Public Health Research, National Institute for Public Health Research, Mexico, and the London School of Hygiene and Tropical Medicine.
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early neonatal deaths. Early neonatal deaths were identified by following up all babies to the seventh day after delivery. To control for the possible confounding effect of birth weight, babies were considered in two groups: normal birth weight (NBW), those weighing ≥2500 gm, and low birth weight (LBW), those weighing <2500 gm. Inasmuch as the early neonatal death rate is high among LBW babies (20%), the case-control approach was not suitable and all LBW babies were included in the study. For NBW babies a case-control method was used; every thirtieth NBW baby born in the study hospitals who survived the early neonatal period was systematically selected as a control. Details of the care received by case and control subjects were obtained by interview with the mother and from review of all medical records. Multiple births, babies who weighed <1000 gm, and those with lethal congenital malformations were excluded from the analyses of the relationship between cesarean section and early neonatal mortality.

To control for the confounding effect of factors other than birth weight an index of perinatal risk was used similar to that of Chamberlain et al.[23] (see Table I). This index was constructed for each mother and the sum of the values of the variables was calculated for each woman. The women were classified as low risk if the sum added to less than three, medium if three to seven, and high for all those with a score above seven. The risk score was able to successfully categorize women into three groups with significantly different outcomes (early neonatal mortality) (see Table II).

For LBW babies the ratio of the mortality rates between exposed and unexposed (to cesarean delivery) was used as the measure of association and for NBW babies the odds ratio of exposure between control and case subjects was used. To control for the confounding effects of many variables (e.g., risk status, parity, years of schooling) in the analysis of the association between early neonatal death and cesarean section, indirect standardization and the Mantel and Haenszel procedure[24] were used for LBW and NBW babies, respectively.

Information from hospital records was used to estimate the whole-time equivalents of interns, residents, and specialist obstetricians working in the maternity wards of the study hospitals.

Results

Over the 14-week period 32,701 babies were born in the 25 hospitals. There were 518 early neonatal deaths for a mortality rate of 16.0 per 1000 live births. Of the 32,329 babies born alive 3103 (9.6%) weighed <2500 gm. Among the LBW babies, 240 early neonatal deaths and 2192 survivors were included and for the NBW babies, 52 deaths and 906 controls were included. The remaining 226 early neonatal deaths were excluded because the birth weight was <1000 gm or they had a lethal congenital malformation.

Table I. Variables and weights* used to construct the perinatal risk score

Variables and category	*Weight**
Age of mother (yr)	
<20 and 30-34	1
20-29	0
>34	2
Parity of mother	
1 and 4	1
2 and 3	0
>4	2
Education of mother (yr)	
0-3	2
4-12	1
>12	0
Previous abortion	4
Previous stillborn	4
Previous neonatal death	4
Previous low birth weight	2
Diabetes	4
Height <150 m	1
Single mother	2

Babies of normal birth weight classified as low risk if score is less than three, medium risk if three to seven, and high risk if over seven.

*Weightings taken from Chamberlain et al.[23]

Over one fourth (27%) of deliveries were by cesarean section and this was more common among women in private hospitals (39%) than those in Social Security (25%) or public assistance (21%). Cesarean delivery was more frequent among LBW (33%) than in NBW babies (23%).

For the LBW babies the early neonatal mortality rate was 36 times higher in the high-risk group as compared with the low-risk group and three times that in the medium-risk group. Among NBW babies the mortality rate was more than three times higher in the high-risk than in the medium-risk group (there was only one death among the NBW low-risk babies) (see Table II). Paradoxically the cesarean section rate was only moderately related to the risk score (high risk, 33%; medium risk, 29%, and low risk, 26%).

In both birth weight groups babies delivered by cesarean section were more likely to die in the early neonatal period. This statistically increased relative risk (RR) of mortality persisted after adjustment for risk status (Table III).

It is difficult to estimate the extent to which the excess of early neonatal deaths among cesarean section, as compared with vaginally delivered babies, was a result of the "medical" condition that prompted the decision to undertake a cesarean section. However, this is unlikely to be confounded by other factors because birth weight was controlled for by stratification into two groups. In addition, the maternal risk score includes

Table II. Early neonatal mortality rates for babies* born to mothers with different perinatal risk scores by two birth weight groups

	Low birth weight				*Normal birth weight*			
Perinatal risk score	*Births*	*%*	*Deaths*	*Early neonatal mortality rate†*	*Births‡*	*%*	*Deaths*	*Early neonatal mortality rate†*
Low	572	23.5	4	6.9	9921	36.5	1	0.1
Medium	1347	55.7	107	79.4	13427	49.4	25	1.9
High	513	21.1	129	251.5	3832	14.1	26	6.8
TOTAL	2432	100.0	240	98.7	27180	100.0	52	1.9

Early neonatal mortality rate by risk groups for both low and normal birth weights p value for X^2 linear trend < 0.0001.

*Only singleton and babies weighing ≥ 1000 gm. Babies with lethal congenital malformations excluded.

†Per 1000 live births.

‡Numbers of normal birth weight births by risk group estimated from complete sample.

Table III. Early neonatal mortality by mode of delivery by birth weight groups

	Mode of delivery	
Birth weight groups	*Vaginal*	*Cesarean section*
Low birth weight		
Early neonatal mortality*	81.1	140.5
Numbers (deaths/births)	139/1713	101/719
Crude relative risk†	1.0	1.73‡
Adjusted relative risk§	1.0	1.54‖
Normal birth weight		
Early neonatal mortality*	1.3	3.7
Numbers (deaths/births)	25/19020	27/7290
Crude relative risk¶	1.00	2.82#
Adjusted relative risk§	1.00	2.51**

*Per 1000 live births.

†Risk ratio.

‡95% Confidence limits 1.36, 2.25.

§Adjusted for maternal risk (see Table I).

‖95% Confidence limits 1.24, 1.86.

¶Odds ratio of exposure with use of data from cases and controls.

#95% Confidence limits 1.60, 4.80.

**95% Confidence limits 1.43, 4.41.

several factors strongly associated with the prevalence of medical conditions taken as indications for cesarean delivery. That the RR did not significantly change after adjustment for maternal risk suggests that mothers were similar in the two groups (cesarean and vaginal deliveries). The implication of this is that the excess of early neonatal mortality in cesarean births cannot be explained by differences in maternal age, parity, education, obstetric history, diabetes, height, and marital status, or probably by differences in medical conditions associated with these factors.

To further explore whether the marked excess of early neonatal mortality among NBW babies was confounded by other factors additional bivariate adjustments of relative risks were undertaken. The results of these analyses are summarized in Table IV.

The adjustment for maternal risk gave a higher RR of early neonatal mortality for cesarean deliveries for medium-risk babies than those in the high-risk group. The maternal age group with the lowest overall risk of early neonatal mortality (20 to 29 years) had the greatest excess associated with cesarean section. The adjusted RR for parity was higher than the crude RR, suggesting that parity has some negative confounding effect; whereas the adjusted RR for gestational age was slightly lower, indicating that some of the excess mortality is probably a result of differences in gestational age between the two groups of babies.

The RR of early neonatal mortality for babies delivered by cesarean section was considerably higher in public assistance (4.76) than in Social Security hospitals (2.07). Although the cesarean section rate was high (39%) in private hospitals, there were no deaths among babies delivered this way. It is widely recognized that a far higher porportion of births in public assistance than in Social Security hospitals are supervised by interns and residents as opposed to specialists. Social Security hospitals have more than 1.5 times the specialists and far fewer interns than do public assistance hospitals (Table V). Unfortunately relative risks of mortality could not be estimated by type of birth attendant because of incomplete information.

The RR of early neonatal mortality for babies delivered by cesarean section from mothers with spontaneous onset of labor was 3.38 [confidence limits (CL), 1.82, 6.27], higher than for those born to mothers whose labor was induced 1.96 (CL, 0.39, 10.10). About 12% of the cesarean deliveries were elective and the RR of early neonatal mortality for elective cesarean section compared with emergency cesarean section was 0.80 (CL, 0.22, 2.85).

For babies who weighed ≥2500 gm, significantly

Table IV. Changes in relative risk of early neonatal mortality for cesarean delivered babies, after controlling for certain variables

Variables	*Relative risk* maximum likelihood*	*95% Confidence limits*
Crude	2.82	1.60, 4.80
Maternal characteristics		
Risk score	2.51	1.43, 4.41
Mother's age	3.19	1.69, 5.99
Mother's education	3.01	1.71, 5.26
Mother's parity	3.04	1.75, 5.27
Fetal position	2.36	1.93, 5.43
Baby's characteristics		
Birth weight	2.69	1.55, 4.67
Gestational age	2.51	1.51, 4.72
Related to quality of care		
Health system	2.94	1.71, 5.06
Induction of labor	3.08	1.75, 5.73

Only normal birth weight babies were included.
*Reference group is vaginally delivered babies.

raised relative risks of early neonatal mortality were found among those delivered by cesarean section. A striking finding was that babies with the lowest absolute risk of early neonatal mortality (those weighing 3000 to 3499 gm) had the highest relative risk of dying in the early neonatal period when delivered by cesarean section. The significantly raised relative risks of early neonatal mortality for babies who weigh ≥2500 gm when delivered by cesarean section persisted even after controlling for major obstetric, social, and biologic risk factors.

The overall attributable risk of early neonatal mortality associated with cesarean delivery, that is the rate of early neonatal mortality among cesarean births minus the rate among vaginally delivered babies, was 13.9 (i.e., 23.1 − 9.2): for LBW babies, 50.4 (i.e., 138.0 − 87.6) and for NBW babies, 4.3 (i.e., 6.5 − 2.2). The etiologic fraction of early neonatal mortality associated with exposure to cesarean delivery was 27%, being lower for the LBW (10%) than the NBW babies (32%).

The relative risk of early neonatal mortality among cesarean-delivered babies compared with those delivered vaginally increased steadily with birth weight except for the heaviest babies (>3500 gm) (see Fig. 1). The largest relative risk was for babies who weighed 3000 to 3499 gm at 5.66 (CL, 2.41, 13.28). These babies, who had the best chances of neonatal survival, account for 42% of all babies born and 38% of all cesarean deliveries. Almost one fourth of the babies in this birth weight group were delivered by cesarean section giving an etiologic fraction of 42% associated with cesarean section.

The cesarean section rate was 87% in breech as opposed to 22% in cephalic presentations. The relative risk of early neonatal mortality by cesarean delivery was 0.30 (CL, 0.05, 1.86) for breech presentations and 3.42 (CL, 1.84, 6.38) for cephalic presentations.

Table V. Designated specialists, residents, and interns (whole-time equivalents) in the maternity wards of hospitals by health system

Type of medical personnel	*Health system*			
	Public assistance		*Social Security*	
	No.	*%*	*No.*	*%*
Designated specialists per 10,000 births	15.6	16.7	24.0	34.9
Residents years 1-3 per 10,000 births	42.7	45.6	33.6	48.9
Interns per 10,000 births	35.0	37.0	11.1	16.2
TOTAL	—	100.0	—	100.0

Ratio of residents and interns to specialists, 2 + 3/1: public assistance, 4.99:1; Social Security, 1.86:1.
Relative risk of early neonatal mortality for cesarean births: public assistance, 4.76; Social Security, 2.07.

Comment

Over the past 20 or so years cesarean section has become an increasingly common form of delivery.[1-9] The apparent safety of the procedure under optimal conditions has led to the suggestion that all women who have not been delivered of infants by term should have a "prophylactic" cesarean delivery.[25] The major concerns about high and increasing rates of cesarean section principally centered around the costs and neonatal morbidity.[21, 26-29]

It is accepted that the procedure is efficacious in certain defined situations, but as with all technology

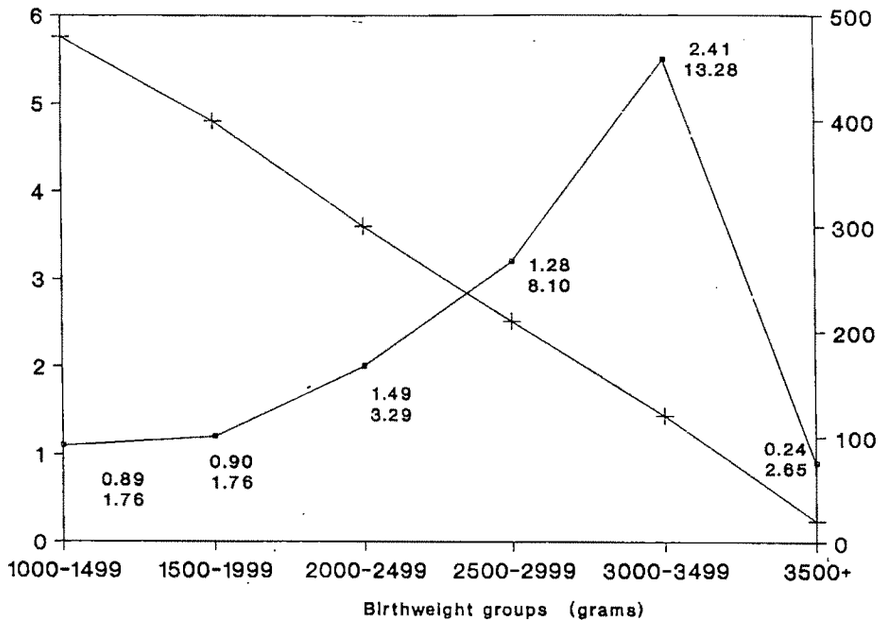


Fig. 1. Early neonatal mortality rate and relative risk of early neonatal death for babies delivered by cesarean section by birth weight group. Right-hand scale is early neonatal mortality rate per 1000 live births. Left-hand scale is relative risk of death for babies delivered by cesarean section compared with vaginally delivered babies; numbers are 95% confidence intervals for relative risk.

its effectiveness should be questioned when used without clear indications and in the absence of quality assurance programs. Recognition of these issues in the United States led the National Institute of Child Health and Human Development to organize a consensus conference on cesarean childbirth in 1980.[30] This concern has been reflected elsewhere with more aggressive efforts to reduce the proportion of deliveries carried out by cesarean section[7] and has been shown to be effective where high standards of care are provided.[31, 32]

Cesarean delivery has been shown to be associated with low Apgar score,[21] and a raised incidence of hyaline membrane disease[28] and respiratory distress syndrome.[27, 29, 33] The main complication among mature NBW babies is delayed onset of respiration.[34] The present study confirms Usher's original work[35] carried out in 1964, which showed an increased risk of respiratory distress syndrome in infants delivered by cesarean section. It is possible that the increased incidence of respiratory distress syndrome and delay in spontaneous respirations among babies delivered by cesarean section is a result of deficient secretion of hormones because of a lack of the physical stress that normally occurs during vaginal delivery.[29, 36-38]

In Mexico City cesarean delivery is relatively common, ranging from 39% of births in large private hospitals to 21% in public assistance hospitals, this is in contrast to the percentages of high-risk babies who were 8% and 18%, respectively. This study showed that it was only in breech presentations that the RR of early neonatal mortality associated with cesarean delivery was lower than that for vaginally delivered babies. Others have reported similar findings.[39, 40]

The association of the health system with the high RR of early neonatal mortality associated with cesarean section in NBW babies in this study was highly significant. This probably reflects the situation with regard to several aspects of quality of care. In the public assistance hospitals the decision to undertake a cesarean section is usually made by a resident in obstetrics with limited training, and support in anesthesia and pediatrics is provided by colleagues also with inadequate experience.

In private hospitals there are obvious financial incentives for obstetricians to resort more readily to cesarean delivery than are justified on strict obstetric criteria.[9] In public hospitals this does not apply, but given the high status of the private sector it is possible that practice there sets a standard that influences the threshold at which cesarean section is thought appropriate. However, there are other factors that encourage high rates of cesarean section: many hospitals are staffed mainly by residents with limited experience, their supervision is frequently inadequate, and it is consequently not surprising that their threshold to carry out a cesarean section is low. It removes the necessity to

monitor the progress of labor and gives obstetricians greater control over the timing of deliveries.

In Mexico City with the absence of quality assurance systems there are limited checks on whether the operation was medically justified. This study suggests that in this situation in which cesarean section rates are high and many cesarean deliveries are carried out under suboptimal conditions with no clear medical indications to deliver infants of normal birth weight with a low overall risk of early neonatal mortality, might result in unnecessary neonatal deaths. In view of this there is a pressing need to define objective criteria for undertaking cesarean sections and to institute review procedures of the circumstances that surround cesarean sections such as those described by Myers and Gleicher[32] and Porreco[11] from the United States.

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The effect of tetracycline on levels of oral contraceptives

Ana A. Murphy, MD,[a] Howard A. Zacur, MD, PhD,[a] Patricia Charache, MD,[b] and Ronald T. Burkman, MD[c]

Baltimore, Maryland

Despite the widespread use of tetracycline for treatment of dermatologic disorders and sexually transmitted diseases, the pharmacodynamics of oral contraceptive use in the presence of tetracycline has not been studied. Seven normal women ingested an oral contraceptive containing ethinyl estradiol 35 μg and norethindrone 1 mg in the follicular phase of the menstrual cycle. On day 0 baseline ethinyl estradiol and norethindrone levels were obtained at 0, ½, ¾, 1, 2, 4, 12, and 24 hours after oral contraceptive administration. On day 1 tetracycline 500 mg was given orally every 6 hours while the oral contraceptive was continued. Tetracycline, ethinyl estradiol, and norethindrone levels were determined at the same time intervals as on day 0. Oral contraceptive and tetracycline were continued for up to 10 days, and additional concentrations of ethinyl estradiol, norethindrone, and tetracycline were determined between days 5 and 10. Four additional normal women ingested tetracycline for 5 to 10 days. Tetracycline levels were determined at the time intervals noted above on day 1 and days 5 to 10. No significant decrease in plasma ethinyl estradiol or norethindrone concentration was seen with either short-term (24 hours) or long-term (5 to 10 days) ingestion of tetracycline. Similarly, levels of tetracycline do not significantly decrease with ingestion of a low-dose oral contraceptive. (Am J Obstet Gynecol 1991;164:28-33.)

Key words: Oral contraceptives, tetracycline, ethinyl estradiol, norethindrone

The controversial topic of drug interaction between oral contraceptives and antibiotics dates back to 1971 when Reimers and Jeyeck[1] noted an increase of abnormal vaginal bleeding in oral contraceptive users receiving rifampin and other antituberculous drugs. Two years later, Nocke-Finck et al.[2] reported that 5 of 88 rifampin–oral contraceptive users became pregnant while 62 of the remaining women experienced abnormal bleeding or amenorrhea. Since then there have been numerous case reports of oral contraceptive users becoming pregnant while receiving ampicillin, tetracycline, chloramphenicol, griseofulvin, sulfonamides, and others.

The postulated mechanism by which broad-spectrum antibiotics interfere with oral contraceptives is alteration of the gut flora so that less estrogen is reabsorbed. It is, however, very uncertain whether broad-spectrum antibiotics such as ampicillin and tetracycline decrease the efficacy of oral contraceptives. Joshi et al.[3] have demonstrated no decrease in area under the curve or in peak or mean levels of the oral contraceptive components when women ingest ampicillin or metronidazole.

Tetracycline is a drug of choice for the outpatient treatment of pelvic inflammatory disease and some other sexually transmitted diseases. Most patients need a 7- to 10-day course of this antibiotic; often times many

From the Division of Reproductive Endocrinology and Infertility,[a] the Division of Infectious Disease,[b] and the Division of Gynecology,[c] Johns Hopkins University.
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Reprint requests: Ana A. Murphy, MD, UCSD Medical Center, Department of Reproductive Medicine, T-002, 225 W. Dickinson St., San Diego, CA 92103.
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are also using oral contraceptives. Although the question of interaction between these two drugs is commonly brought up, there are no studies that definitively address this point. Only case reports suggest possible interaction.[4] The purpose of this study is to determine whether tetracycline affects contraceptive steroid levels. The effect of oral contraceptives on tetracycline levels also will be assessed.

Material and methods

Subjects. Seven healthy, normally menstruating women aged 18 to 35 were studied. None had received steroid treatment within the last 3 months before the study. The study protocol was approved by the internal review board and informed consent was obtained from each volunteer. An oral contraceptive containing 35 μg of ethinyl estradiol and 1 mg of norethindrone was begun within 1 to 5 days of the onset of the menstrual cycle. Serum samples for ethinyl estradiol and norethindrone were obtained on day 0 between 7 and 9 AM, before oral contraceptives were begun, and ½, ¾, 1, 2, 4, 12, and 24 hours after ingestion of the oral contraceptive. The next day (day 1), at the same time the oral contraceptive pill was ingested the previous day, another oral contraceptive pill was ingested in conjunction with 500 mg of tetracycline hydrochloride (Lederle Laboratories, Pearl River, N.Y.). The tetracycline was continued at that dose every 6 hours. Serum samples for ethinyl estradiol, norethindrone, and tetracycline were drawn at the same time intervals cited. The patients continued to ingest the oral contraceptive pill each morning and tetracycline every 6 hours. Five to 10 days later, serum samples were obtained at the time intervals indicated. One patient failed to finish the study because of gastrointestinal side effects.

Four healthy, normally menstruating women aged 18 to 35 were studied as tetracycline controls. None had received steroid treatment within the 3 months before the study. Tetracycline hydrochloride 500 mg was begun within 1 to 5 days of the onset of the menstrual cycle. The tetracycline was continued every 6 hours for 5 to 10 days. Serum samples for tetracycline were obtained before the antibiotic was begun and at the time intervals noted previously for ethinyl estradiol, norethindrone, and tetracycline. The sampling was repeated 5 to 10 days later. One patient failed to finish this part of the study because of gastrointestinal effects. All sera were stored at −20° C until analysis.

Assay methods. Ethinyl estradiol and norethindrone were quantified by direct radioimmunoassay with a modification of the procedures described by Stanczyk et al.[5, 6] Tritiated ethinyl estradiol and norethindrone were obtained from New England Nuclear (Boston). The ethinyl estradiol antibody was obtained from C. Edgar Cook (Research Triangle Institute, Research Triangle Park, N.C.). The norethindrone antibody was graciously donated by Ing Nieuweboer (Schering AG, Berlin). The intraassay coefficient of variation was 10.6% for ethinyl estradiol and 11.6% for norethindrone. The interassay coefficient of variation was 18.7% for ethinyl estradiol and 12.5% for norethindrone. The sensitivity of the assay was 12 pg/ml for ethinyl estradiol and 0.15 ng/ml for norethindrone.

Tetracycline serum samples were assayed by an agar well diffusion assay.[7, 8] Two milliliters of a 1:100 dilution of *Bacillus cereus* spore suspension (Difco Lab., Detroit) and 23 ml of antibiotic Medium 1 (Difco) for each 150 mm polystyrene petri plate were used.

Standards and controls were prepared with tetracycline hydrochloride standard powder (Lederle), which was reconstituted in sterile deionized water and diluted in heat-inactivated pooled normal human serum (Whittaker M.A. Bioproducts, Walkersville, Md.) to yield the following standard concentrations: 1, 2, 4, 8, and 16 μg/ml. A control serum sample containing 6 μg/ml of tetracycline was prepared in 100 ml aliquots, stored at −70° C, and thawed on the day of use.

Five-microliter samples of standard, control, or patient serum were placed into 3 mm wells in the bioassay plate. Standards were run in duplicate; control and patient sera were run in triplicate assays per plate. The plates were incubated overnight at 37° C and the zones of inhibition were measured. Concentrations for patients were determined from a plot of the log of the standard concentration vs the zone diameters (in millimeters). The standard curve correlation of coefficients ranged from 0.990 to 1.000. Coefficient of variation for the 6 μg/ml control serum was 7.7%. Therapeutic levels of tetracycline are 2 to 4 μg/ml.

Statistical methods. Repeated measures analysis of variance and covariance was used for analysis of the effects of tetracycline on ethinyl estradiol and norethindrone levels (BMDP Statistical Software, Inc., Los Angeles). Area under the 4-hour curve was calculated with RSI software (BBN Software Products, Cambridge, Mass.) and peak levels were compared at days 0, 1, and 5 to 10. Multiple range testing was done once significance was reached, with analysis of variance. The subject who did not complete the study was not included in the analysis.

The effects of oral contraceptives on tetracycline levels were examined by a two-way analysis of variance. Tetracycline 4-hour area under the curve of the patients using oral contraceptives and tetracycline (day 1 and days 5 to 10) were compared with the control subjects who received tetracycline alone (day 1 and days 5 to 10). Peak levels of tetracycline in these two groups were compared by means of the same statistical test.

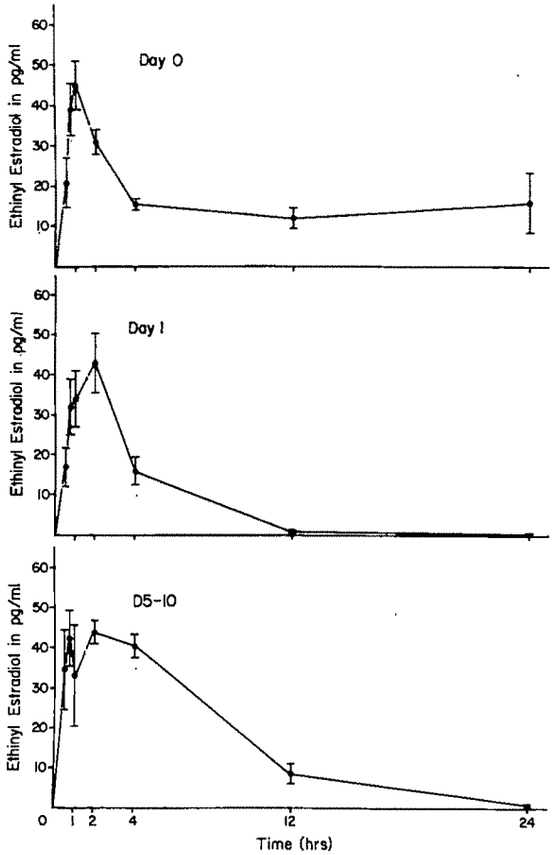


Fig. 1. Twenty-four-hour plasma levels of ethinyl estradiol on pill day 0 (oral contraceptive only) and on day 1 and days 5 to 10 after ingestion of tetracycline and oral contraceptive. Values are mean ± SEM.

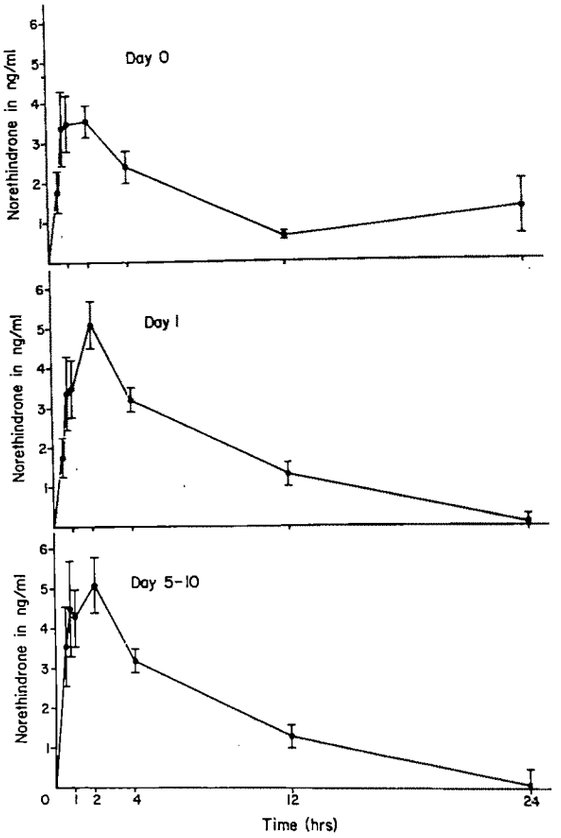


Fig. 2. Twenty-four-hour plasma levels of norethindrone on pill day 0 (oral contraceptive only) and on day 1 and days 5 to 10 after ingestion of tetracycline and oral contraceptive. Values are mean ± SEM.

The peak levels are reported as mean ± SEM. The subject who did not complete the study was included in the analysis.

Results

The mean (± SEM) 24-hour curves of ethinyl estradiol and norethindrone at day 0, day 1, and days 5 to 10 are shown in Figs. 1 and 2. The mean 24-hour area under the curve for ethinyl estradiol alone demonstrated no significant difference when compared with that for day 1 and days 5 to 10 (Table I). Similarly the mean peak value of ethinyl estradiol on day 0 was not significantly different from values on day 1 or days 5 to 10.

The mean 24-hour area under the curve for norethindrone demonstrated a statistically significant difference when day 0, day 1, and days 5 to 10 are compared ($p < 0.0007$) (Table I). Multiple range analysis by time revealed a significant increase between day 0–day 1 and days 5 to 10. The mean peak levels of norethindrone on day 0, day 1, and days 5 to 10 were significantly different ($p < 0.01$). Multiple range testing again revealed the increase to be between day 0–day 1 and days 5 to 10.

Four-hour area under the curve values for tetracycline in those subjects ingesting the oral contraceptive pill on day 1 and days 5 to 10 were not significantly different from those of subjects receiving tetracycline alone on day 1 or days 5 to 10 (Table II). Similarly, the peak levels of tetracycline did not differ from those of the oral contraceptive–tetracycline group on day 1 and day 5 or the tetracycline group on day 1 and days 5 to 10 (Table II).

Comment

The changes observed for 24-hour area under the curve for ethinyl estradiol and norethindrone demonstrate that oral contraceptive levels are not decreased by concomitant ingestion of tetracycline, either short-term (24 hours) or long-term (5 to 10 days). This is not

Table I. Effect of tetracycline in plasma levels of ethinyl estradiol and norethindrone in women ingesting oral contraceptive and tetracycline starting on day 1

	24 hr area under curve		*Peak plasma level*	
Pill day	*Ethinyl estradiol*	*Norethindrone*	*Ethinyl estradiol (pg/ml)*	*Norethindrone (ng/ml)*
0	1057.2 ± 151.3	26.4 ± 2.7*	101.5 ± 24.1	4.1 ± 0.5†
1	1054.3 ± 95.4	33.0 ± 4.5*	91.4 ± 18.3	4.5 ± 0.5†
5-10	1114.4 ± 121.3	57.9 ± 6.5*	98.2 ± 15.9	6.3 ± 1.0†

Values are mean ± SEM.
*$p < 0.0007$.
†$p < 0.01$.

Table II. Effect of oral contraceptive steroids on plasma levels of tetracycline in women ingesting tetracycline and oral contraceptive and in women ingesting tetracycline alone

	Tetracycline			
	4 hr area under curve		*Peak plasma level (mg/ml)*	
Day	*Tetracycline and oral contraceptive*	*Tetracycline only*	*Tetracycline and oral contraceptive*	*Tetracycline only*
1	10.6 ± 1.5	14.6 ± 1.0	5.9 ± 1.0	5.1 ± 0.8
5-10	24.8 ± 5.9	26.7 ± 2.1	7.5 ± 1.9	7.6 ± 0.6

Values are mean ± SEM.

surprising since the only available data implicating tetracycline are from case reports that are uncontrolled, retrospective, and subject to recall bias.

The evidence cited for a potential clinically important broad-spectrum antibiotic and steroid interaction is based on several studies showing a reduction in urinary estriol excretion and lower serum estriol levels in pregnant women taking ampicillin.[9-12] This antibiotic is thought to alter the gastrointestinal metabolism of estrogens by decreasing the bacterial flora possessing glucuronidase and sulfatase enzymes, which are necessary for hydrolysis of conjugated steroids. This process is essential for reabsorption of estrogens because the conjugated steroids are poorly resorbed. The result is increased fecal loss of the conjugated steroids (estrogens and ethinyl estradiol in particular), thus reducing plasma levels of the steroid metabolites. This alteration in enterohepatic circulation is not relevant to the progestin component of oral contraceptives because only inactive progestin metabolites can be obtained after hydrolysis.[13]

The clinical importance of gastrointestinal metabolism of contraceptive steroids should be questioned.[14] The bioavailability of ethinyl estradiol and levonorgestrel does not appear to be reduced in women with an ileostomy.[15] Since the vast majority of glucuronidase activity in the human occurs in the large bowel, this study in women with ileostomy suggests that the enterohepatic recycling of contraceptive steroids may not be clinically significant in humans. Moreover, human studies do not suggest that contraceptive steroid levels are affected by treatment with ampicillin or metronidazole.[3, 16, 17] The ethinyl estradiol curve on day 0 does not suggest that the enterohepatic circulation is important in determining plasma levels of ethinyl estradiol. However, although we sampled over 24 hours, there are relatively few points on the curves between 4 and 24 hours. There may be secondary peaks, perhaps as a result of enterohepatic circulation, which we failed to detect.

Our data are the first to demonstrate that tetracycline does not decrease the peak plasma levels or the area under the curve for ethinyl estradiol. Our study included prospective follow-up of the subjects, therefore we were able to note the relatively large intersubject variability in plasma ethinyl estradiol levels with relatively small intrasubject variability. However, because of our study design (repeated measures analysis of variance), our ability to detect a statistical difference (power of the test) is much greater than with study designs that use unpaired groups. Failure to detect a difference is probably not a type II error; however, because of the relatively small number of subjects in the study, small differences would probably not be detected.

Our data demonstrate that norethindrone peak levels

and area under the curve did not decrease with either short-term (24 hours) or long-term (5 to 10 days) ingestion of tetracycline. Interestingly, both of the parameters evaluated showed a statistically significant increase between day 0–day 1 levels and levels on days 5 to 10.

Back et al.[18] report that the plasma half-life of the terminal, apparently monoexponential phase of norethindrone decay was between 6.1 and 12.3 hours in six subjects ingesting a single dose of 1 mg norethindrone and 50 μg ethinyl estradiol. During the long-term therapy with oral contraceptive pills, the plasma half-life did not differ. However, in four subjects receiving oral contraceptive pills for the first time, plasma concentrations of norethindrone rose progressively during the first cycle to concentrations higher than predicted from simple accumulation pharmacokinetics.[19] The sex-hormone-binding-globulin–binding capacity also increased, and there was a good correlation between the plasma concentration of norethindrone and the sex-hormone-binding-globulin–binding capacity. We have demonstrated a significant increase in sex-hormone-binding-globulin levels in patients ingesting a low-dose oral contraceptive containing 1 mg of norethindrone and 35 μg of ethinyl estradiol.[20] It is possible that levels of norethindrone may have been even higher with the oral contraceptive alone than with oral contraceptive pills and tetracycline on day 1 and days 5 to 10. These data are not available in our study. It is unlikely, however, that such is the case since a previous study with frequent, 24-hour sampling at 6 to 7 days of oral contraceptive (1 mg norethindrone, 30 mg ethinyl estradiol) use does not show an increase in norethindrone levels.

There is no evidence that the enterohepatic metabolism of progesterone or its metabolites is of any great physiologic importance in health or disease.[14] Moreover, although there are no data on the enterohepatic circulation of progestins in humans, it appears that in rats the bioavailability of progestins is not affected by changes in the enterohepatic circulation.[21] However, an effect of tetracycline on the enterohepatic circulation cannot be excluded.

The area under the 4-hour curve and peak levels of tetracycline do not appear to be decreased by oral contraceptives. Peak levels of tetracycline ranged from 5.1 to 7.9 μg/ml. In vitro studies indicate that concentrations above 2 to 4 μg/ml are therapeutic. Thus the results of our study suggest that the therapeutic efficacy of tetracycline is not reduced by the concurrent ingestion of a combination low-dose oral contraceptive. Similar results have been obtained with ampicillin and metronidazole.[3]

We have demonstrated for the first time that peak and 24-hour area under the curve for norethindrone and ethinyl estradiol do not appear to decrease in subjects ingesting tetracycline on either a short-term (24 hours) or a long-term (5 to 10 days) basis. While we have not examined pregnancy rates, unchanged or increased contraceptive steroid levels would suggest that the efficacy of the oral contraceptive is not reduced. Additionally, plasma levels of the antibiotic do not appear to be decreased in the presence of an oral contraceptive. Thus it would suggest that the therapeutic efficacy of tetracycline is not decreased.

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Venous Doppler ultrasonography in the fetus with nonimmune hydrops

Saemundur Gudmundsson, MD, PhD,[a] James C. Huhta, MD,[a] Dennis C. Wood, RDMS, RCPT,[a] Gerald Tulzer, MD,[a] Arnold W. Cohen, MD,[b] and Stuart Weiner, MD[c]

Philadelphia, Pennsylvania

Eighteen pregnancies with nonimmune hydrops fetalis were referred for fetal echocardiography to rule out congenital heart disease. In 14 of these cases, pulsating blood velocities were recorded in the umbilical vein, which in a normal population had a nonpulsatile blood velocity pattern. The four cases without pulsations in the umbilical vein were found to have intrauterine viral infections. In the last 10 cases examined, the umbilical venous pulsations were found to reflect abnormal central venous pulsations during atrial systole suggesting increased fetal central venous pressure. Right ventricular shortening fraction was significantly decreased in the group with umbilical venous pulsations compared with those without (0.18 versus 0.32, $p < 0.05$). All the fetuses without venous pulsations survived, but only four of the 14 with pulsations survived ($p < 0.05$). The results suggest that blood velocity recordings in the umbilical and central veins of the fetus can give valuable clinical information with regard to the presence of fetal congestive heart failure and differentiate between this physiologic state and other causes of nonimmune hydrops fetalis. This may have implications for fetal diagnostic work-up and prognosis. (Am J Obstet Gynecol 1991;164:33-7.)

Key words: Fetus, nonimmune hydrops, Doppler ultrasonography, venous pulsations, congestive heart failure, umbilical vein

Nonimmune hydrops fetalis is a term applied to a fetus with excessive fluid accumulation in fetal body cavities along with soft tissue edema when fetomaternal blood group incompatibility is absent. Nonimmune hydrops fetalis has many causes,[1] but in many cases the common underlying pathophysiologic mechanism, whether primary or secondary, is heart failure.[2] In other cases of nonimmune hydrops fetalis, congestive heart failure may not be present. Noninvasive physiologic methods that define congestive heart failure in the fetus are not currently available but Doppler ultrasonography can provide blood velocity correlates of abnormal physiology. Umbilical venous pressure has been found to be increased in fetuses with congestive heart failure and hydrops from fetal anemia during cordocentesis[3] but there are no reliable noninvasive correlates of this hemodynamic state. Blood velocity fluctuations in the inferior vena cava have been reported as a correlate of central venous pressure changes in animal studies.[4]

While fetuses referred for echocardiography because of nonimmune hydrops fetalis were studied, abnormal blood velocity pulsations in the umbilical vein were noted. Our goals in this study were (1) to evaluate

From the Division of Cardiology, Children's Hospital of Philadelphia, University of Pennsylvania,[a] and the Departments of Obstetrics and Gynecology, Hospital of the University of Pennsylvania[b] and Pennsylvania Hospital.[c]
Received for publication August 1, 1990; accepted August 3, 1990.
Reprint requests: James C. Huhta, MD, Division of Cardiology, Children's Hospital of Philadelphia, 34th and Civic Center Blvd., Philadelphia, PA 19104.
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whether the pulsating pattern could differentiate between cardiac and noncardiac involvement in nonimmune hydrops fetalis; (2) to investigate whether the abnormal pulsations were caused by atrioventricular valve insufficiency; and (3) to determine whether the venous blood velocity pattern could predict the fetal outcome of pregnancy.

Material and methods

Eighteen pregnant women with fetuses that had ultrasonographic evidence of nonimmune hydrops fetalis were referred for echocardiographic studies to rule out congenital heart disease. The mean age was 29 years (range, 19 to 38), five being nullipara, nine primipara, and four multipara. The mean gestational age was 29 weeks (range, 23 to 36). All the fetuses had fluid collections in two or more body compartments. Ascites was observed in all cases, 15 had pleural effusion, eight had pericardial effusion, and six had skin edema. Polyhydramnious was present in nine cases and oligohydramnious in one case. All fetuses had evaluation for fetal infection consisting of blood viral titers before or after delivery.

Six of the fetuses were the recipients in twin-to-twin transfusion as judged by discordant body size and only the hydropic twin in these pregnancies was analyzed. Four fetuses had supraventricular tachycardia and the assessment was performed during tachycardia. Five fetuses had viral infections documented by either positive immunoglobulin M titers (two with cytomegalovirus and one with parvovirus), and two with positive immunoglobulin G for cytomegalovirus and parvovirus, respectively. In another case the hydrops disappeared within 3 weeks, suggesting a viral cause. No cause of the hydrops was defined in three cases. Another fetus had a congenital heart malformation (a form of hypoplastic left heart syndrome with valvular regurgitation) and another had a placenta with multiple chorioangiomas.

A 3.5 MHz pulsed Doppler ultrasonogram in combination with a 3.5 MHz ultrasonographic sector scanner (Acuson 128) was used to record the umbilical artery blood flow velocity waveforms from a central portion of the umbilical cord. All recordings were performed without fetal breathing or body movements. The waveforms recorded were analyzed for pulsatility index according to Gosling et al.[5] and related to normal reference values.[6] Blood velocity was simultaneously recorded in the umbilical vein. All measurements were taken in the absence of fetal breathing. Normal umbilical vein blood velocity was affected by fetal breathing and abnormal venous pulsations are illustrated in Fig. 1. In the last 10 cases examined, blood velocities were also recorded from the fetal inferior vena cava between the entrance of the renal veins and the ductus venosus and the criteria of Reed et al.[11] were used to define significant reversal. All fetuses had 3.5 MHz color Doppler ultrasonography to locate the vessels and to search for atrioventricular insufficiency jets, which then were recorded by 3.5 MHz continuous-wave Doppler ultrasonography (Acuson 128). Doppler examinations were performed at least weekly in all patients before delivery but only the first examination at the time of diagnosis of hydrops was analyzed. The mean time interval between examination and delivery was 17 days (range, 0 to 62). The results of the fetal Doppler recordings were not available to the clinicians managing the patients. M-mode recordings were performed of the right ventricle in 16/18 fetuses and the shortening fraction was calculated as the diastolic minus the systolic dimension divided by the diastolic dimension.

Outcome was evaluated at review of newborn records for gestational age at delivery; birth weight[7]; delivery by cesarean section; intrauterine fetal death (death before or during birth); and neonatal death (up to 4 weeks after birth). Fisher's exact test (two-tailed) was used for statistical evaluation of the results. Parametric variables were compared with the unpaired two-tailed Student t test.

In addition, the umbilical vein velocity was recorded by the same method in 27 normal pregnant hospital staff volunteers after informed consent was obtained. A total of 51 examinations was performed between 20 and 40 weeks' gestation. All fetuses had a complete echocardiographic examination to exclude any evidence of congenital heart disease and a normal postnatal follow-up echocardiogram within the first 3 weeks after birth.

Results

Normal pregnancies. In our group of normal pregnancies, umbilical vein velocity was found to be even and without any fluctuations (except during fetal breathing movements) (Fig. 1) between 20 and 40 weeks' gestation. Whereas normal umbilical cord venous velocity is constant, fluctuating venous velocities were found to be normal in the peripheral veins such as the renal vein.

Nonimmune hydrops fetalis group. Eleven of the eighteen patients were delivered by cesarean section, two of which were indicated for fetal distress by fetal monitoring. The mean gestational age at delivery was 32 weeks (range, 23 to 41). The mean birth weight was 2022 gm (range, 560 to 4010).

Pulsating blood flow velocity in the umbilical vein was recorded in 14 of the 18 cases with nonimmune hydrops fetalis. In the four cases without pulsations in the umbilical vein, causes of the nonimmune hydrops fetalis were documented viral infections in three and viral infection was suspected in one. The fetal hydrops

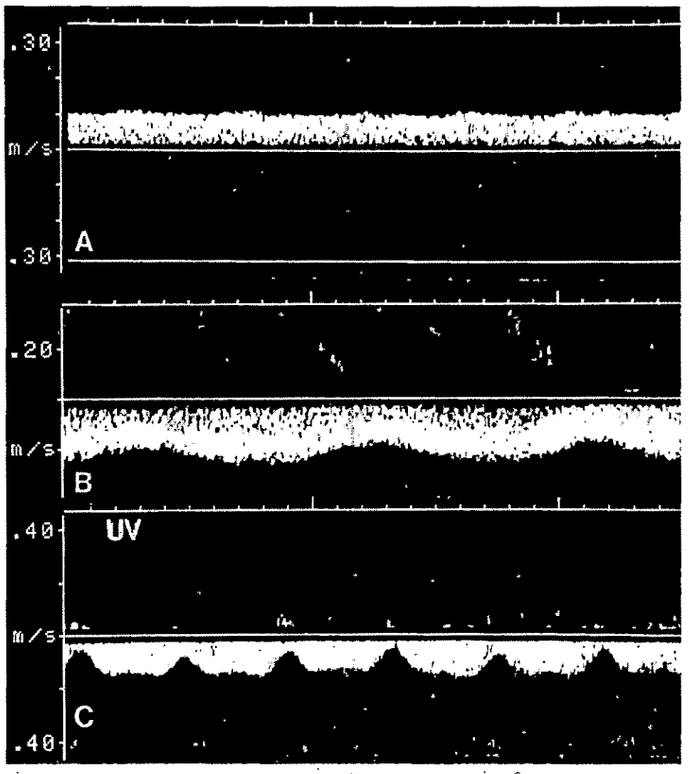


Fig. 1. Blood velocity in fetal umbilical vein. **A,** Normal blood velocity pattern; **B,** blood velocity influenced by fetal breathing; and **C,** venous pulsations in nonimmune hydrops.

resolved before delivery in these four cases; in one case fetal hydrops resolved after an intravenous fetal blood transfusion for anemia (parvovirus). The other three had mild hydrops that resolved within 21 days of diagnosis without treatment. Hydrops was present at delivery in 13 of the 14 cases with umbilical vein pulsations, but in none of the fetuses without umbilical vein pulsations. The one case in which umbilical vein pulsations were found before delivery and hydrops had disappeared by the time of delivery was a fetus that had supraventricular tachycardia that was successfully treated in utero. There was a significant difference in total mortality (10/14, 71%) between the group with venous pulsations and without (0.4, $p < 0.025$ (Table I). There were five intrauterine deaths that all had abnormal umbilical vein pulsations.

In 10 cases blood velocities were also recorded in the inferior vena cava and significant reversal was found in all with umbilical pulsations. The time velocity integral of the reversed flow in the inferior vena cava averaged 46% of the forward flow (range, 33% to 56%) compared with a normal maximum of 7% in our normal group. A normal and abnormal inferior vena cava blood velocity recording is illustrated in Fig. 2.

The umbilical artery blood velocity waveform pulsatility index was normal in all except two cases that had absent end-diastolic velocity. The mean pulsatility index ± SD values for both groups was not significantly different (Table I). The presence of valvular regurgi-

Table I. Results at echocardiographic examination in fetuses with nonimmune hydrops with and without pulsations in the umbilical vein

	Umbilical vein pulsations (n = 14)	*No pulsations (n = 4)*	*p Value*
Gestational age at examination (wk)	29*	31†	<0.375
RV SF	0.18 ± 0.11	0.32 ± 0.13	<0.025
Umbilical artery PI	1.33 ± 0.6	1.03 ± 0.1	<0.375
TR	8	1	–
IUFD	5	0	–
Neonatal mortality	5	0	–
Total mortality	10	0	<0.025

TR, tricuspid regurgitation; *IUFD,* intrauterine fetal death; *PI,* pulsatility index; *RV SF,* right ventricular shortening fraction. Mean values ± SD or range are given.

*Range, 23 to 36.

†Range, 23 to 35.

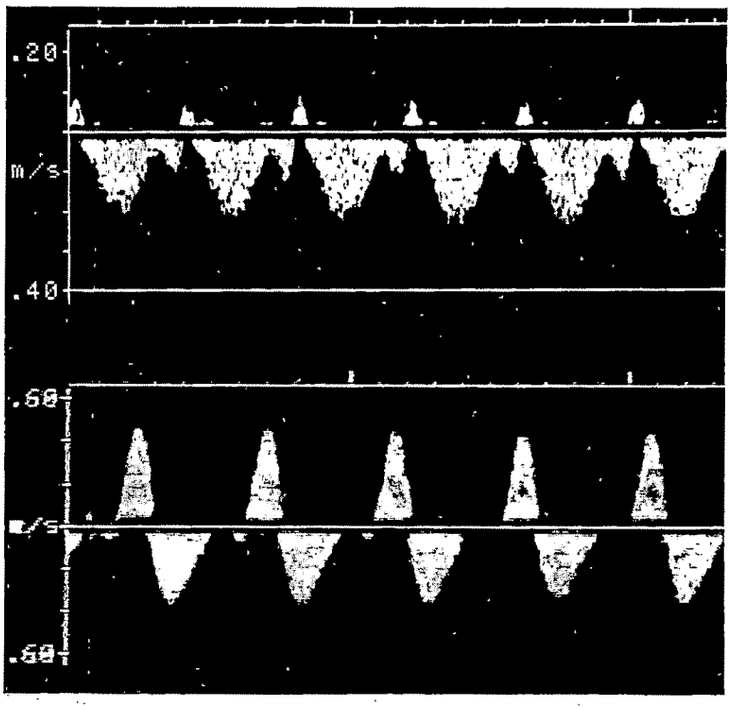


Fig. 2. Blood velocity in fetal inferior vena cava. *Above,* normal blood velocity pattern and *below,* reversal of venous flow in nonimmune hydrops.

tation between fetuses with normal and abnormal umbilical venous blood velocities was not different although 8/14 had tricuspid regurgitation ($p < 1.22$). Right ventricular shortening fraction was significantly decreased in the group with umbilical venous pulsations compared with those without (0.18 versus 0.32, $p < 0.05$).

Comment

Doppler velocities in the umbilical artery give important physiologic information concerning the placental impedance and may aid in the management of high-risk pregnancies. However, umbilical venous Doppler velocities have not been used to evaluate fetal well being. In normal pregnancies we found that Doppler-recorded blood velocity in the umbilical vein is even and without fluctuation after 20 weeks' gestation. It is also our experience that the fetal inferior vena cava, renal, hepatic, axillary, and cerebral veins have pulsating blood velocities in normal pregnancy. Fourteen of the 18 fetuses with nonimmune hydrops fetalis in the present series had abnormal pulsations in the umbilical vein; the four fetuses without pulsations had proved or suspected viral causes. In the last ten cases examined, central venous blood velocities were also recorded and exaggerated fluctutations in velocities were present with reversal of flow away from the heart during end-diastole, which all exceeded the normal range.[8] Abnormal reversal of blood velocities in the central veins extended beyond the ductus venosus into the umbilical vein. These observations of an abnormal pressure wave during atrial systole point to cardiac involvement in the disease process. The finding of decreased ventricular shortening in those with pulsations suggests chronic congestive heart failure. Pulsations were not absolutely correlated with valvular regurgitation probably because the degree of myocardial dysfunction necessary to yield regurgitation depends on the cause of hydrops. Only one patient had hydrops because of a malformed valve. The importance of the finding of valvular regurgitation by Doppler ultrasonography in a hydropic fetus remains unclear. Nonetheless, venous pulsations were timed to atrial systole and were not correlated to valvular regurgitation.

An increase in capillary membrane permeability as a part of an inflammatory reaction might be the pathophysiologic mechanism behind the hydrops in the viral cases and gives hope that Doppler recordings of blood velocities in the central veins can distinguish between congestive heart failure and such a cause of nonimmune hydrops fetalis. A congenital infection may cause liver damage and hypoproteinemia as another possible mechanism for nonimmune hydrops fetalis, and myocarditis in such a fetus could result in congestive heart failure. Two of the fetuses in the group with viral infection (Table I) had pulsations in the umbilical vein;

both showed a decreased right ventricular shortening fraction and tricuspid regurgitation.

All the fetuses without pulsating blood velocities in the umbilical vein survived compared with only four of the 14 with abnormal pulsations. This might be explained partly by differences in gestational age at birth (Table I), but the hydrops had disappeared at delivery in all the cases without pulsations and in only one of the cases with pulsations—a case of supraventricular tachycardia that was successfully treated. The four caess with pulsations that survived had suspected viral causes of nonimmune hydrops fetalis (two) or supraventricular tachycardia (Table I). Absence of abnormal pulsations in the venous system, apart from being able to aid in the diagnosis of congestive heart failure with nonimmune hydrops fetalis, thus might also predict fetal outcome.

The umbilical artery blood velocity waveform was of limited value in the prediction of the fetal outcome of pregnancy. Two cases had absent end-diastolic blood velocities and all the others had normal umbilical artery pulsatility indexes even after correcting the values in cases with tachycardia according to Mires et al.[9] We speculate that umbilical artery flow velocity waveform does not reflect the disease state as in intrauterine growth retardation,[10] perhaps because significant elevations in placental or systemic vascular resistances are not necessarily a part of the pathogenesis of nonimmune hydrops fetalis.

Pulsating blood velocities in the umbilical vein have also been noted in human beings where this phenomenon was suggested as being a sign of a dysfunctional ductus venosus.[11] More likely, they reflect dynamic changes in venous flow similar to those in this group of patients. Reversal of end-diastolic blood flow in the fetal inferior vena cava has been reported to be associated with asphyxia in animal studies,[4] in human fetuses with α-thalasemic hydrops,[12] and in fetuses with arrhythmia or the absence of end-diastolic blood velocity in the umbilical artery.[8] The blood velocity waveform in the inferior vena cava was defined as abnormal when the time-velocity integral of the reversal of blood flow during atrial systole was >12% of the forward blood flow during one heart cycle. The reversal of blood velocities in the inferior vena cava in our series were all beyond this limit.

In conclusion, flow in the umbilical vein in normal pregnancies is constant in the absence of fetal breathing movements and pulsations in the umbilical vein are a reflection of central hemodynamics. Umbilical and central venous Doppler ultrasonography can give important information on the filling function of the heart and may be able to distinguish between fetuses with and without congestive heart failure that have nonimmune hydrops fetalis. Umbilical venous pulsations are not a direct result of tricuspid valve regurgitation but either sign appears to reflect cardiac involvement in nonimmune hydrops fetalis. The absence of pulsations in the umbilical vein may be a useful prognostic sign in the fetus with nonimmune hydrops fetalis.

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The relationships among umbilical artery velocimetry, fetal biophysical profile, and placental inflammation in preterm premature rupture of the membranes

Alfred D. Fleming, MD, Carolyn M. Salafia, MD, Anthony M. Vintzileos, MD, John F. Rodis, MD, Winston A. Campbell, MD, and Kimberly F. Bantham, BS
Farmington, Connecticut

The relationships among umbilical artery velocimetry, fetal biophysical profile, and placental inflammation in 44 consecutive patients with preterm premature rupture of the membranes were determined. All patients were followed up with daily fetal biophysical profiles and systolic/diastolic ratios. After delivery, placental pathologic examination for histologic evidence of infection (umbilical vasculitis) was performed in all cases according to a standard protocol. The longitudinal trends of fetal biophysical profile scores and systolic/diastolic ratios were analyzed for patients with and without umbilical vasculitis. Analysis of the longitudinal trend during the period of 2 to 7 days before delivery showed that there were no differences of biophysical scores of systolic/diastolic ratios in either group. However, in the last examination, within 24 hours of delivery, patients with umbilical vasculitis had higher systolic/diastolic ratios and lower biophysical profile scores as compared with previous examinations. In addition, the biophysical profile score of the last examination 24 hours before delivery in patients with umbilical vasculitis was found to be significantly lower as compared with patients without umbilical vasculitis (mean ± SD, 6.6 ± 2.3 versus 8.2 ± 2.4, respectively). These data suggest that subclinical stages of infection are associated with biophysical alterations of the fetoplacental unit (i.e., simultaneous decrease in fetal biophysical activities and increase in systolic/diastolic ratios). Daily umbilical artery systolic/diastolic ratios, in conjunction with biophysical profiles, may be useful to follow up patients with preterm premature rupture of the membranes. (Am J Obstet Gynecol 1991;164:38-41.)

Key words: Umbilical artery velocimetry, fetal biophysical profile, placental inflammation, preterm premature rupture of the membranes

Acute intraamniotic infection is a major contributor to maternal and neonatal morbidity and mortality. This is because of the association of acute intraamniotic infection with preterm labor and maternal and neonatal sepsis. In our institution, patients with preterm premature rupture of the membranes (PROM) are followed up with daily fetal biophysical profiles. Patients in whom abnormal biophysical assessment develops are delivered promptly.[1] This approach is undertaken on the basis of our previous observations that suggested that abnormal biophysical assessment in patients with PROM precedes the development of overt clinical infection. In such patients, the fetal biophysical assessment is abnormal in the absence of fetal acidosis.[2] The cause of diminished fetal biophysical activities without blood acidosis has been related to the hemodynamic changes that accompany an increased production of prostaglandins from phospholipase A_2–producing bacteria. Because there are experimental data to suggest that prostaglandins increase placental vascular resistance through vasoconstriction of the umbilical and chorionic vessels,[3,4] it would be reasonable to investigate the role of umbilical arterial velocimetry in this group of patients.

The aims of the present prospective study were to test the hypothesis that the placental vascular resistance, as determined by umbilical artery velocimetry, is increased in patients with placental inflammation and to assess the relationships among umbilical artery velocimetry, fetal biophysical profile, and placental inflammation in patients with preterm PROM.

Material and methods

The study group consisted of 44 consecutive patients admitted with preterm PROM from July 1, 1988, to June 30, 1989. All patients were transported to the University of Connecticut Health Center from regional hospitals. All patients were middle to upper socioeco-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Connecticut Health Center.
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Reprint requests: Alfred D. Fleming, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The Perinatal Center, Suite 4810, Creighton University, Omaha, NE 68131.
6/1/24760

Table I. Longitudinal trends of fetal biophysical profile scores

	Days before delivery			
	4-7	*3*	*2*	*1*
Group 1				
No inflammation (N = 17)	10.2 ± 1.5	9.2 ± 1.6	9.1 ± 1.8	8.2 ± 2.4
Group 2				
Umbilical vasculitis (N = 22)	10.1 ± 1.1*	9.9 ± 1.4*	9.4 ± 1.4*	6.6 ± 2.3

Values are mean ± SD.
*p Value < 0.05 (as compared with the last examination 1 day before delivery).

Table II. Longitudinal trends of S/D ratios

	Days before delivery			
	4-7	*3*	*2*	*1*
Group 1				
No inflammation (N = 17)	2.9 ± 0.24	2.7 ± 0.31	2.5 ± 0.45	2.6 ± 0.57
Group 2				
Umbilical vasculitis (N = 22)	2.8 ± 0.55	2.6 ± 0.40*	2.4 ± 0.32*	2.8 ± 0.55

Values are mean ± SD.
*p Value < 0.05 (as compared with the last examination 1 day before delivery).

nomic class. Of the 44 patients, 40 were white and four were black. Patients with clinical amnionitis or bleeding on admission were excluded. Patients were also excluded if other complicating factors such as pregnancy-induced hypertension, diabetes, or intrauterine growth retardation were present.

All patients had rupture of the membranes diagnosed by sterile speculum examination with pooled fluid, ferning, alkaline pH determination. Bimanual pelvic examinations were not performed unless the patient was in labor or induction of labor was planned. On admission to the hospital, maternal vital signs were recorded and electronic fetal heart rate monitoring was used to detect signs of fetal distress and document any uterine activity. In the absence of labor, fetal distress, or obvious clinical infection, ultrasonographic examination was performed to assess gestational age and to determine the fetal biophysical profile. The fetal biophysical profile was determined by a technique previously described.[5] The maximum score was 12 and the minimum 0. The ultrasonographic examination was performed by means of linear-array real-time ultrasonography (General Electric SRT 2600, Wilmington, Mass.) with a 3.5 MHz transducer. An abnormal biophysical assessment (score <7 on two examinations 2 hours apart in the presence of a nonreactive nonstress test and absence of fetal breathing) was used as an indication for delivery. Umbilical artery systolic/diastolic (S/D) ratios were also performed on admission by means of continuous-wave Doppler (Multigon 500A, Mt. Vernon, N.Y.). Three separate ratios were attained and the mean value recorded similar to the method described by Schulman et al.[6]

All patients were managed in the hospital with bed rest, bathroom privileges, daily biophysical profiles, and daily umbilical S/D ratios. The results of the umbilical artery velocimetry did not influence patient management. Temperatures were taken four times a day and white blood cell counts were performed daily. Increased white blood cell count alone was not used as an indication for delivery. Daily white blood counts were performed to aid in the diagnosis of clinical amnionitis; however, none of the patients had clinical infection during the antepartum period. Indications for delivery included abnormal biophysical profile, fetal distress on electronic fetal monitoring, or labor. Indications for cesarean section included history of previous cesarean section, fetal malpresentation, ominous heart rate pattern on internal fetal monitoring, and failure to progress. In patients with abnormal biophysical assessment, if the fetus was in vertex presentation and the fetal heart rate tracing reassuring (normal short-term variability by internal monitoring), induction of labor by oxytocin administration was undertaken.

Placental pathologic examination was performed in all patients according to standard protocol.[7] Placentas were weighed after removal of adherent blood clot and umbilical cord detachment. Two sections of umbilical cord, a section of membrane from the site of rupture, a membrane roll, and a section of chorionic plate with two chorionic vessels were examined from each patient.

Microscopic diagnoses were assigned by a single observer (C.M.S.) blinded to the biophysical scores and S/D ratio results.

In accordance with the placental pathologic findings, two groups were identified: group 1 had no histologic evidence of infection on maternal or fetal surfaces of the placenta; group 2 had histologic evidence of infection involving fetal polymorphonuclear leukocytes (umbilical, chorionic vasculitis). Patients with intermediate degrees of inflammation involving only maternal polymorphonuclear leukocytes (without umbilical-chorionic vasculitis) were also collected.

Statistical analysis of categoric variables (e.g., presence or absence of umbilical vasculitis) were performed with the use of contingency tables (Fischer's exact probability test). Analysis of continuous variables was performed by analysis of variance. Analysis of variance of repeated measures was used to check for significant daily changes between measurements (biophysical profile scores and S/D ratios). A p value <0.05 was considered statistically significant.

Results

The mean gestational age of the 44 fetuses was 29 weeks, with a range of 24 to 34 weeks. The cesarean section rate was 25/44 (57%). The indications for cesarean sections were repeat cesarean section (5 or 20%), fetal malpresentation (7 or 28%), nonreassuring fetal heart rate pattern on external monitoring with a closed cervix precluding internal monitoring (6 or 24%), ominous heart rate pattern on internal fetal monitoring (5 or 20%), and failure to progress (2 or 8%). Placental histologic examination resulted in the assignment of 17 patients to group 1 (no inflammation), and 22 to group 2 (umbilical vasculitis present). There were only five patients with placental inflammation confirmed only to the maternal surface (choriodeciduitis-chorionitis) and therefore they were excluded from further analysis. The mean gestational age of group 1 was 29.6 weeks (range, 24 to 33.5 weeks) and of group 2 was 29.0 weeks (range, 24 to 34 weeks). In group 1, delivery was indicated because of abnormal biophysical assessment in 4 of 17 (24%), labor in 11 of 17 (65%), and fetal distress in 2 of 17 (12%). In group 2 abnormal biophysical assessment was reported in 17 of 22 (77%) and labor in 5 of 22 (23%). The frequency of delivery for abnormal biophysical assessment was significantly higher in group 2 (77% vs 24%, $p < 0.05$).

The biophysical score and S/D ratio results according to placental infection outcome are shown in Tables I and II, respectively. During the period of 2 to 7 days before delivery, there were no differences between the two groups in terms of biophysical scores and S/D ratios. However, in the last examination within 24 hours of delivery, group 2 was found to have significantly lower biophysical scores as compared with group 1 (6.6 ± 2.3 vs 8.2 ± 2.4, respectively, $p < 0.05$). The longitudinal trend of the biophysical scores and S/D ratios results for each of the two groups revealed a significant decrease in the mean biophysical profile score of group 2 in the last examination within 24 hours before delivery, as compared with the previous examinations. Biophysical scores of patients in group 1 did not differ significantly from day to day including the last examination before delivery. In group 1 S/D ratios did not show significant differences in values observed on successive days before delivery.

In group 2 the mean S/D ratio of the last examination within 24 hours of delivery was significantly elevated as compared with previous examinations. The mean increase of S/D ratio values observed in the last examination before delivery in group 2 was 22% over previous examinations (range, 0% to 74%). An increase of >15% in S/D ratio would have a sensitivity of 63%, specificity of 73%, positive predictive value of 71%, and negative predictive value of 61% for prediction of acute umbilical vasculitis.

Comment

This study confirms the clinical association between abnormal biophysical profile assessment and histologic evidence of infection as determined by placental pathology. This study also suggests that the longitudinal trend in S/D ratio values may be an additional noninvasive means to identify fetuses at risk of ascending intrauterine infection with preterm PROM. The increase in S/D ratios observed longitudinally in fetuses from group 2 in the last 24 hours before delivery coincide with the decrease in biophysical score over the same time period. The increase in umbilical vascular resistance and diminished biophysical score supports a role for vasoactive substances (e.g., prostaglandins) as mediators of infection causing vasoconstriction of umbilical and chorionic vessels.[3, 4] This increase in vascular resistance may compromise uteroplacental circulation and diminish biophysical activities. Changes in fetal heart rate patterns have already been linked to the presence of histologic evidence of intraamniotic infection.[8, 9]

These observations further support the value of following up longitudinally the trends of both biophysical score and S/D ratio in patients with PROM. It might be that a decreasing trend in biophysical score and an increasing trend in S/D ratio values might increase the detection rate of fetuses at risk of infection. The fact that none of our patients had any clinical evidence of infection during the examinations suggests that the biophysical profile and S/D ratio become abnormal in subclinical stages of intraamniotic infection.

It should be emphasized that the mean S/D ratio in

the last examination within 24 hours before delivery in cases with umbilical vasculitis although elevated as compared with previous daily examinations is within normal limits according to previously established nomograms.[6] Other studies of the association of changes in S/D ratio with PROM have not described abnormal S/D ratios in this setting.[10] This is likely a result of two factors. First, the significant elevation of S/D ratio that was observed in the present study was only after follow-up of the longitudinal trend. Second, this elevation was confined to patients with placental infection. This emphasizes the importance of serial evaluation of S/D ratio in the setting of preterm PROM. Although umbilical cord gas values were obtained in all cases, no attempt was made in this study to correlate them with the biophysical assessment because we have previously shown that no such correlation exists in the presence of fetal infection.[2]

In summary, the present study provides pathologic evidence of the clinical association between abnormal biophysical assessment and impending infection in patients with preterm PROM. This study also suggests that umbilical artery velocimetry may have a role as a noninvasive means to follow up fetuses at risk of ascending intrauterine infection in patients with preterm PROM. Further studies are needed to determine whether the use of umbilical artery velocimetry can be an additional means to assess fetuses with preterm PROM to thereby reduce maternal and neonatal infectious morbidity and mortality.

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Human immunodeficiency virus–induced immunosuppression: A risk factor for human papillomavirus infection

Roberto Matorras, MD, PhD,[a] Jose M. Ariceta, MD,[a] Angel Rementeria, MD,[a] Jesús Corral, MD,[b] Gloria Gutierrez de Terán, RN,[a] Javier Diez, MD,[a] Francisco Montoya, MD, PhD,[a] and Francisco J. Rodriguez-Escudero, MD, PhD[a]

Bilbao, Spain

In a group of 92 women with genital condylomata, 15 (16.3%) human immunodeficiency virus–positive patients were found, whereas no case was detected in a control group of 100 women. The relative risk was >19.28. Human immunodeficiency–positive status was associated with other parameters: lower age and parity, major frequency of induced abortions, and sexually transmitted diseases. Thus although human immunodeficiency–positive status seems to be a true risk factor in relation to the altered immunologic state, an indirect association cannot be discarded. Such patients should be screened closely for human papillomavirus infection and cervical cancer. Among human immunodeficiency–positive women, a more resistant behavior of human papillomavirus–associated lesions was detected (recurrence-persistence of 41.7% versus 12%), a fact that might also be in relation to the immunodepressed status. (AM J OBSTET GYNECOL 1991;164:42-4.)

Key words: Human immunodeficiency virus, acquired immunodeficiency syndrome, human papillomavirus, recurrence, persistence

Human papillomavirus (HPV) association with cervical cancer is well known. In HPV infection the immunologic status plays an important role. It has been reported that during states of altered immunity such as pregnancy[1] and in immunosuppressed patients there is an increased risk of HPV infection.[2] Interferon efficacy has been proved in the therapy of resistant condyloma.[3]

There are reports that the human immunodeficiency virus (HIV) infection might be associated with the HPV infection.[4] If this statement is confirmed, such patients should be screened closely for HPV infection, and therefore for cervical cancer.

Material and methods

Between 1986 and 1989 the diagnosis of HPV infection was established in 92 patients at the Colposcopy Unit of the Oncological Gynecology Service of the Hospital of Cruces, Pais Vasco University School of Medicine in Spain. The diagnosis was made on the basis of the presence of genitoperineal warts or a directed cervical biopsy specimen with evidence of HPV infection. The main epidemiologic features of this population have been reported recently.[5]

From the Department of Obstetrics and Gynecology[a] and the Department of Microbiology,[b] Hospital de Cruces (Baracaldo), School of Medicine, Pais Vasco University.
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Reprint requests: Dr. R. Matorras, Jon Arrospide 24, 7D, Bilbao, Spain 48014.
6/1/24839

Papanicolaou smear, colposcopy, and colposcopically directed biopsies were performed in the usual way. Cytologic criteria for diagnosis of HPV infection were parakeratosis, koilocytosis and dyskaryosis. Colposcopic study was classified in three groups: (1) condylomatous (raised, irregular lesions); (2) atypical (abnormal lesions that were acetowhite and flat), and (3) normal (without colposcopic anomalies). Histologic features considered diagnostic of HPV infection were koilocytosis, dyskeratosis, multinucleation, and hyperkeratosis.

A randomized control group was established that comprised 100 women without pathologic findings who were attended in the same unit and whose macroscopic inspection, cervical cytology, and colposcopy showed no signs of HPV infection. In doubtful cases, directed biopsies were performed. Specific methods to detect HPV were not used in either the study group or in the control group.

In our Maternal Hospital the HIV antibodies detection test was performed during the same period in women with a history of intravenous drug abuse, prostitution, and partners of intravenous drug abusers or HIV-positive males. HIV antibodies were analyzed by means of enzyme-linked immunosorbent assay and confirmed by Western blot analysis and indirect immunofluorescence. HIV evolutive stage was studied by means of the determination of antigen p24 and antibodies anti-p24 by means of enzyme-linked immunosorbent assay.

Therapy consisted mainly of cryotherapy or laser therapy for cervical condylomata, whereas laser ther-

Table I. Personal history

	Group A: Condylomatous HIV-positive women (n = 15)	*Group B: Condylomatous non-HIV–positive women (n = 77)*	*Group C: Noncondylomatous non-HIV–positive women (n = 100)*	*p Value*		
				A/B	*A/C*	*B/C*
Age	22.6 ± 3.02	29.85 ± 20.56	39.01 ± 12.43	<0.01, S = 2.88	<0.001, S = 11.18	<0.001, S = 3.40
Deliveries	0.2 ± 0.41	0.56 ± 0.89	1.73 ± 1.39	<0.05, S = 2.46	<0.001, S = 8.71	<0.001, S = 6.69
Abortions	0.13 ± 0.35	0.26 ± 0.62	0.25 ± 0.59	NS	NS	NS
Induced abortions	0.67 ± 0.81	0.05 ± 0.23	0.06 ± 0.37	<0.05, S = 2.88	<0.05, S = 2.83	NS
History of STD	53.3%	28.4%	5.0%	0.06, F	<0.001, C = 16.5	0.001, F
Without anticonception	40.0%	47.0%	57.0%	NS	NS	NS

S, The Student *t* test; *C*, χ^2 test; *F*, Fisher's exact test; *STD*, sexually transmitted disease.

apy or local podophyllin were used in vulvoperineal lesions. There were no differences in the indications of different therapies among HIV-positive women and non-HIV–positive women.

The χ^2, Student *t* test, and Fisher exact test were used for the statistical analysis, following the standard criteria of applicability. The relative risk was determined by means of the odds ratio and its limits of confidence. When in one of the groups the number of cases was 0, the odds ratio was established considering 1 as the number of cases, and considering the odds ratio as more than the value determined this way.

Results

Prevalence. Among the 92 patients with condylomata, there were 15 HIV-positive women, representing 16.3% versus no HIV-positive case in the control group ($\chi^2 = 15.6$, $p < 0.001$, odds ratio >19.28, confidence limits >2.58 and >97.84). Among the 15 HIV-positive women there were 13 intravenous drug abusers, whereas in the remaining two patients the mechanism of acquisition was not known. In one patient there was evidence of acquired immunodeficiency syndrome and cerebral toxoplasmosis.

Personal history. Among condylomatous HIV-positive women the age and the number of deliveries were significantly lower than those in condylomatous non-HIV–positive women (Student *t* test = 2.88, $p < 0.01$ and Student *t* test = 2.46, $p < 0.05$, respectively) and those in the control group (Student *t* test = 11.18, $p < 0.001$ and Student *t* test = 8.71, $p < 0.001$, respectively). The age and the number of deliveries were also significantly lower in condylomatous non-HIV–positive women than those in the control group (Student *t* test = 3.40, $p < 0.001$ and Student *t* test = 6.69, $p < 0.001$), respectively.

Among condylomatous HIV-positive women the number of induced abortions was higher than in condylomatous non-HIV–positive women (Student *t* test = 2.88, $p < 0.05$) and the control group (Student *t* test = 2.83, $p < 0.05$). There were no differences between condylomatous non-HIV–positive women and the control group.

Condylomatous HIV-positive women and condylomatous non-HIV–positive women had a higher rate of sexually transmitted diseases than that in the control group (Fisher's exact test = 0.0001, $\chi^2 = 16.49$, $p < 0.001$). The differences between HIV-positive and non-HIV–positive condylomatous women with regard to sexually transmitted disease were close to the statistical significance limit (Fisher's exact test = 0.06).

Clinical findings. Ten of 15 HIV-positive women were first seen with vulvoperineal condylomata (66.7%). In 12 women cervical cytology gave evidence of HPV infection associated or not with different degrees of cervical intraepithelial neoplasia. There was grade 1 cervical intraepithelial neoplasia in two patients, grade 2 cervical intraepithelial neoplasia in three, and grade 3 cervical intraepithelial neoplasia in four, whereas in one patient condyloma was the only finding. In two patients cytologic study results were normal and in another patient such a study was not performed.

Colposcopy gave evidence of condyloma in five patients and results were atypical in seven (in one patient dysplasia-carcinoma was suspected). Colposcopic study found no anomalies in three patients.

Cervical biopsy. In 10 patients the cervix was studied histologically. The histologic diagnoses on the basis of the most severe change were condyloma without atypia in three patients, and in five patients condyloma with different degrees of cervical intraepithelial neoplasia (one grade 1, three grade 2, and one grade 3). In two women, cervical biopsy specimens were normal.

Evolution. Sixty-two patients were followed up for ≥6 months. Among the 12 HIV-positive women, there was persistence or recurrence of HPV-associated lesions in five (41.7%) versus six of the 50 non-HIV–positive women (12%) ($p = 0.03$, Fisher's exact test). Seven HIV-positive women were serologically followed up. One of them was the woman with acquired immunodeficiency syndrome and cerebral toxoplasmosis. In two other patients decreasing antibody levels (anti-

p24) and increasing p24 antigen levels were detected. The remaining four patients were antibody-positive and antigen-negative.

Comment

There are several facts that relate HPV infection to the immunologic status, such as the increased risk in immunodepressed patients[2] and the major frequency[1] and rapid growth during pregnancy,[6] as well as interferon efficacy in the therapy of resistant condyloma.[3] However, only recently an association between HPV- and HIV-induced immunosuppression has been reported. Henry et al.[4] described four HIV-positive women with HPV infection and different degrees of cervical intraepithelial neoplasia.

In our experience 15 HIV-positive women (13 of them heroin addicts) were found to have a clinically detectable HPV infection either in the vulvoperineal area, the cervix, or both. It must be kept in mind that HIV-positive women were significantly younger, with fewer children, and with more induced abortions than those of the control group and those of women with condylomata but who are non-HIV–positive. Sexually transmitted diseases were also significantly increased versus the control group and levels were close to the statistical significance limit versus condylomatous non-HIV–positive women. Thus in our series it was not possible to determine whether the HIV-positive status was a true risk factor or an indirect association. In our study, clinically undetectable HPV infection was not analyzed in the control group. Thus it cannot be discarded that the HIV-positive status could promote the clinical manifestation of latent infections.

Anyway, among HIV-positive women the prevalence of HPV-associated lesions was markedly increased (relative risk, >19.28), justifying a close screening for HPV and cervical cancer in these patients. In our opinion any patient who is an intravenous drug abuser with an HPV infection should have an HIV test, as well as patients with histories of multiple persistence or recurrence of HPV-associated lesions after therapies.

On the other hand, the more resistant behavior of HPV-associated lesions among HIV-positive women could also be in relation to the immunodepressed status. In accordance with this, Carson et al.[2] suggested that patients with multiple recurrent condylomas and genital neoplasias may have underlying immunodeficiency. More data are needed to determine the value of interferon in such patients and to state whether the increased resistance to therapy causes an increased oncogenic transformation.

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Endometriosis associated with massive ascites and absence of pelvic peritoneum

R. Stan Williams, MD, and Rebecca Wagaman, MD
Gainesville, Florida

Although massive ascites associated with endometriosis has been reported in rare cases, this patient was also noted to have massive destruction of the pelvic peritoneum. Failure of medical suppression necessitated total abdominal hysterectomy and bilateral salpingo-oophorectomy. Several months after surgery ascites resolved, possibly with reestablishment of the pelvic peritoneum. (AM J OBSTET GYNECOL 1991;164:45-6.)

Key words: Endometriosis, ascites, peritoneum

Massive ascites associated with endometriosis is rare, having been described in fewer than 20 patients in the English literature.[1] This is a case report of a patient who presented with massive ascites associated with endometriosis; she was also found to have complete destruction of the overlying pelvic peritoneum.

Case report

A 27-year-old black woman, para 0, presented to the Shands Emergency Room at the University of Florida complaining of increasing abdominal girth and shortness of breath for the past 3 months. Endometriosis had been diagnosed by laparoscopy at another hospital 2 years before this presentation. At that time she had complaints of pelvic pain, dysmenorrhea, and dyspareunia. She was treated with a 9-month course of Danocrine postoperatively and her pain resolved with this therapy. She underwent a repeat laparoscopy after completion of Danocrine therapy because of persistent palpable pelvic endometriosis. Endometriosis was again confirmed. Postoperatively, the patient developed a large incarcerated left diaphragmatic hernia and underwent a left thoracotomy. Pathologic examination of the excised diaphragmatic tissue confirmed focal endometriosis with fibrosis. The patient did well until approximately 4 months after surgery when she began to complain of rapidly increasing abdominal girth and subsequent development of a ventral incisional hernia.

From the Department of Obstetrics and Gynecology, University of Florida.
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Reprint requests: R. Stan Williams, MD, Chief, Division of Reproductive Endocrinology and Infertility, Box J-294, JHMHC, Gainesville, FL 32610.
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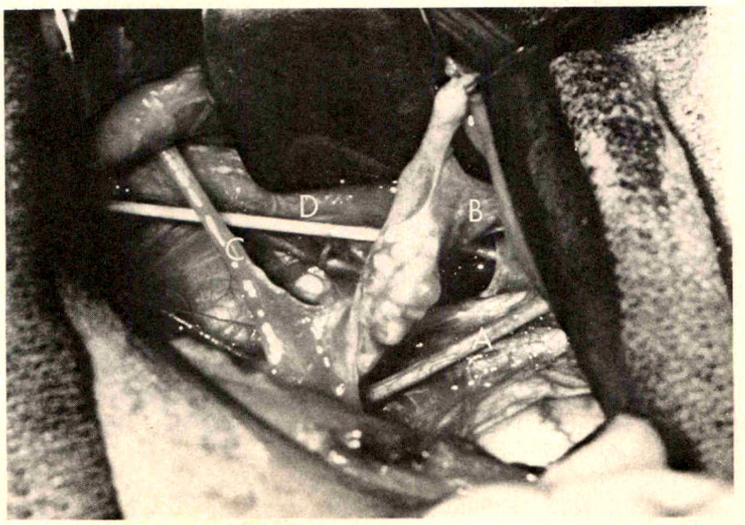


Fig. 1. At laparotomy complete absence of pelvic peritoneum and adventitia was noted with ureters, vessels, and nerves exposed. *A*, Ureter. *B*, Infundibulopelvic vessels. *C*, Round ligament. *D*, Obturator nerve.

She underwent repair of the ventral hernia, at which time a large amount of ascitic fluid was drained.

Evaluation of her massive ascites included negative bacterial and fungal cultures, and normal results of cytologic tests, computerized tomographic scan, complete blood count, electrolyte levels, liver function tests, renal function tests, serum albumin levels, and total protein level. In light of the previous diagnosis of endometriosis, it was thought that ascites was secondary to endometriosis. The patient was begun on a regimen of suppressive therapy with medroxyprogesterone acetate. After 1 month of therapy, the ascites failed to resolve further. She was then treated with depot Lupron (TAP Pharmaceuticals, North Chicago) injection and over 3 months lost an additional 10 pounds of fluid, but she still had a significant amount of ascites. The patient requested definitive surgery.

At the time of laparotomy for total abdominal hysterectomy and bilateral salpingo-oophorectomy she had complete absence of the pelvic parietal peritoneum (Fig. 1). Seven and one-half liters of ascitic fluid was drained. The ureters, blood vessels, and nerves coursed along the pelvic sidewall completely unenveloped by peritoneum or adventitia. The only evidence of gross endometriosis at this time was some scarring in the posterior cul-de-sac overlying the sigmoid colon. Pathologic evaluation confirmed endometriosis in the uterine serosa. One month after operation, there was no evidence of reaccumulation of ascites. Over the next 3 months the patient experienced reaccumulation of ascites and underwent a repeat ventral hernia repair, at which time a Tenchoff catheter was placed. After drainage for 2 weeks, the Tenchoff catheter was removed. Over the past 9 months, there has been no evidence of reaccumulation of ascites.

Comment

This report represents not only the rare association of endometriosis and massive ascites but, in addition, a new finding of complete disappearance of the pelvic peritoneum in association with endometriosis and massive ascites. Although it is recognized that small circular peritoneal defects can be associated with endometriosis,[2] this is the first report of the massive destruction of the entire pelvic peritoneum by endometriosis. Although this patient has not undergone subsequent laparotomy or laparoscopy since the ascites has resolved, it is possible that with the resolution of endometriosis, the normal intraperitoneal mechanisms for healing peritoneum have resulted in the reperitonealization of the pelvis.

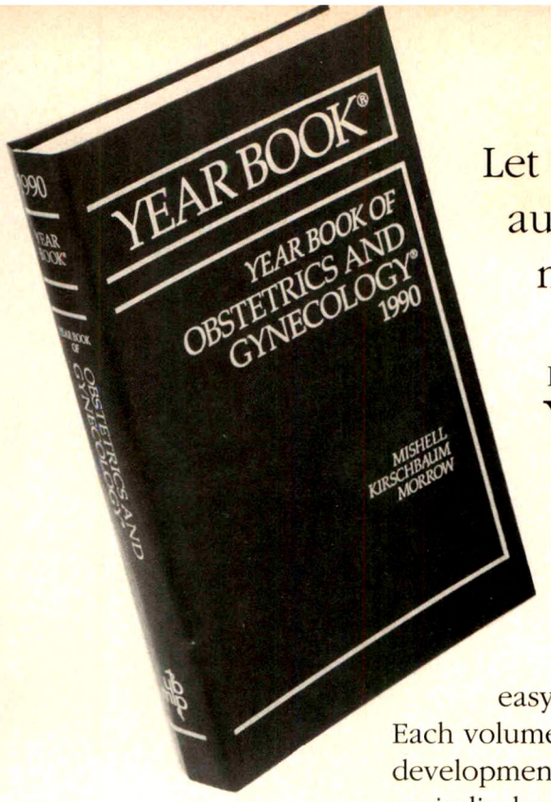
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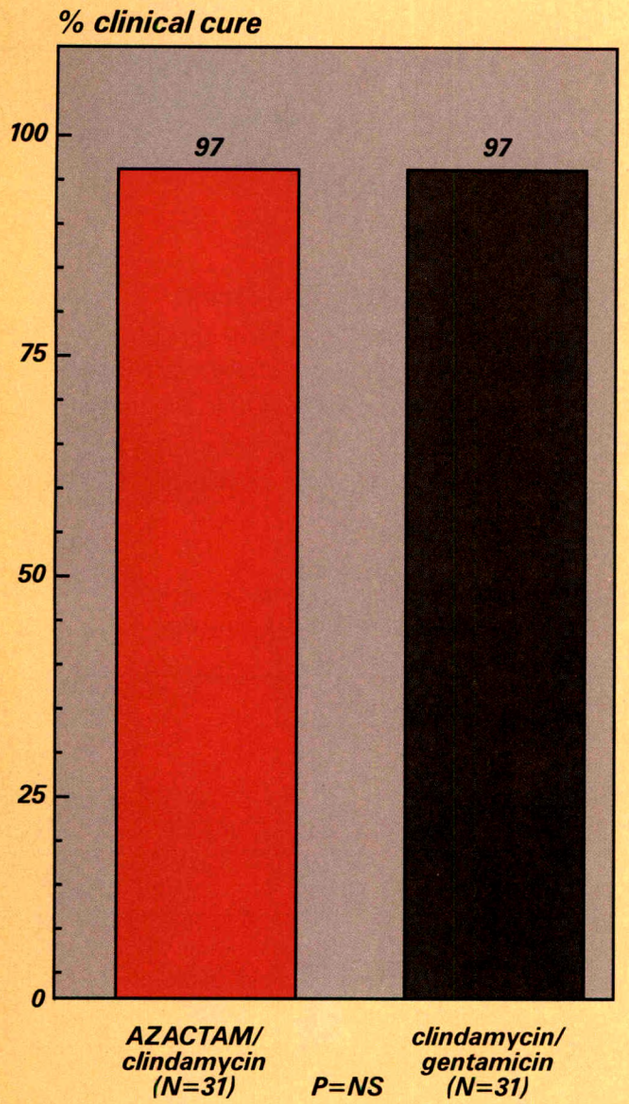
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A randomized, comparative trial of AZACTAM versus gentamicin, each with clindamycin, for the treatment of endometritis after delivery.[1]

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[1]Although a useful guide, in vitro data do not necessarily correlate with therapeutic outcome.

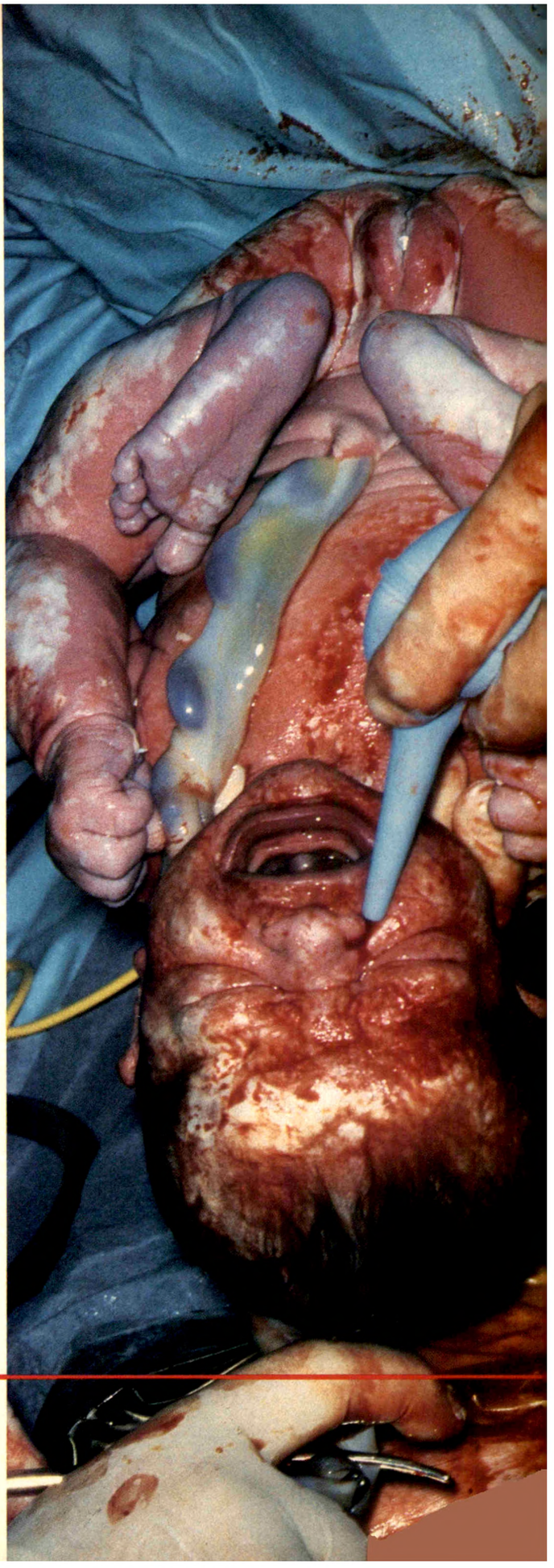
[‡]Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. See full prescribing information for recommendations on patients with immediate hypersensitivity reactions to penicillins.

Following IV administration, the most commonly seen adverse reactions occurred in less than 2% of patients, including local reactions, diarrhea, nausea/vomiting, and rash.

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- *May be used in penicillin-allergic patients.‡*
- *Pregnancy Category B.*

There are no adequate and well-controlled studies in pregnant women. AZACTAM should be used in pregnant women only if clearly needed.



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AZACTAM® FOR INJECTION
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DESCRIPTION–AZACTAM (aztreonam, Squibb) is the first member of a new class of antibiotics classified as monobactams. AZACTAM is a totally synthetic bactericidal antibiotic with activity against a wide spectrum of gram-negative aerobic pathogens. The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics.

AZACTAM For Injection is a sterile, nonpyrogenic, sodium-free, white to yellowish-white lyophilized cake, containing approximately 780 mg arginine per gram of aztreonam for intramuscular or intravenous use following constitution. Aqueous solutions of the product have a pH in the range of 4.5-7.5.

INDICATIONS AND USAGE–Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: **Urinary Tract Infections** (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca*, Citrobacter* species* and *Serratia marcescens**. **Lower Respiratory Tract Infections,** including pneumonia and bronchitis caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens**. **Septicemia** caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*, Serratia marcescens** and *Enterobacter* species. **Skin and Skin-Structure Infections,** including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Citrobacter* species*. **Intra-abdominal Infections,** including peritonitis caused by *Escherichia coli, Klebsiella* species, including *K. pneumoniae, Enterobacter* species including *E. cloacae*, Pseudomonas aeruginosa, Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**. **Gynecologic Infections,** including endometritis and pelvic cellulitis caused by *Escherichia coli, Klebsiella pneumoniae*, Enterobacter* species* including *E. cloacae*,* and *Proteus mirabilis**.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy–Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organisms(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an antianaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS–Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS–Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin). Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

PRECAUTIONS–General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility–Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy–Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers–Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use–Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS–Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity; *Hypersensitivity*–anaphylaxis. *Hematologic*–pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. *Gastrointestinal*–abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). *Dermatologic*–purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. *Cardiovascular*–hypotension, transient ECG changes (ventricular bigeminy and PVC). *Respiratory*–one patient experienced flushing, chest pain, and dyspnea. *Hepatobiliary*–hepatitis, jaundice. *Nervous System*–seizure, confusion, vertigo, paresthesia, insomnia, dizziness. *Musculoskeletal*–muscular aches. *Special Senses*–tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. *Other*–vaginal candidiasis, vaginitis, breast tenderness. *Body as a Whole*–weakness, headache, fever, malaise.

Adverse Laboratory Changes–Those reported without regard to drug relationship during clinical trials were: *Hepatic*–elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*–increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Renal*–increases in serum creatinine.

OVERDOSAGE–If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION–Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg,** or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231C)

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

References: ***1.*** *Greenberg RN, Reilly PM, Weinandt WJ, et el: Comparison trial of clindamycin with aztreonam or gentamicin in the treatment of postpartum endometritis. Clin Ther 10(1):36-39, 1987.* ***2.*** *Gibbs RS, Blanco JD, Lipscomb KA, et al: Aztreonam versus gentamicin, each with clindamycin, in the treatment of endometritis. Obstet Gynecol 65:825-829, 1985.* ***3.*** *Henry SA: Overall clinical experience with aztreonam in the treatment of obstetric-gynecologic infections. Rev Infect Dis 7(suppl 4):S703-S708, 1985.*

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Endometrial thickness as measured by endovaginal ultrasonography for identifying endometrial abnormality

Seth Granberg, MD, Mats Wikland, MD, Bengt Karlsson, MD, Anders Norström, MD, and Lars-Gösta Friberg, MD

Göteborg, Sweden

Diagnostic curettage has for many years been the method of choice to diagnose endometrial cancer in women with postmenopausal bleeding. The costs for curettage performed today are huge, and approximately only 10% in this group of women will be diagnosed with endometrial cancer. Thus less expansive techniques to obtain endometrial samples have been evaluated, but all of them are invasive. The value of endovaginal ultrasonography for identifying endometrial abnormality in this group of women has not been evaluated until now. This study used endometrial thickness as measured by endovaginal ultrasonography as an indicator of endometrial abnormality. It was demonstrated in 205 women with postmenopausal bleeding that if the endometrium was <9 mm thick, no endometrial cancer was found at curettage. The mean endometrial thickness in those women with endometrial cancer was 18.2 ± 6.2 mm as compared with 3.4 ± 1.2 mm in those women with atrophic endometrium. If the cutoff limit for endometrial abnormality was 5 mm, the positive predictive value for identifying endometrial abnormality was 87.3%. If this limit had been used in this study, 70% of the curettage procedures could have been avoided. (AM J OBSTET GYNECOL 1991;164:47-52.)

Key words: Endometrium, endovaginal ultrasonography, endometrial cancer

Postmenopausal bleeding has always been an absolute indication for curettage. Curettage was introduced as early as 1843 by Recamier but came to be a diagnostic procedure in the 20th century. The technique carries a small but real risk of morbidity and death.[1] Furthermore, curettage causes a great deal of anxiety to the patient. With the knowledge that almost 70% of diagnostic curettages result in a diagnosis of a benign condition, an improved "preoperative" evaluation could reduce the number of curettages and bring down the costs considerably.[2, 3] Many different methods have been developed to minimize the need for curettage.[1, 2] One such method is Vabra aspiration (Milex Products, Inc., Chicago). Compared with curettage, it is more convenient since there is no need for general anesthesia, thereby minimizing the risk to women.[1] However, the diagnostic accuracy, when compared with that of curettage, has been debated.[4] This is one reason the use of these methods so far has not significantly influenced the number of curettages performed in women with postmenopausal bleeding.

The histopathologic diagnosis obtained at curettage is used as the gold standard for distinguishing between benign and malignant endometrium. However, the false-negative rate is 2% to 6%.[1, 4-6] This is probably due to the fact that curettage is no guarantee that the uterine cavity has been sufficiently emptied.[6] Thus it is not surprising that some studies have found almost 6% of cases of hyperplasia and cancer were missed at curettage performed before hysterectomy.[4]

Diagnostic ultrasonography is a very good instrument for evaluation of endometrial growth in menstruating women.[7, 8] It is well known that the phasic variation in endometrial thickness can be easily visualized by ultrasonography.[7, 8] However, our knowledge of endometrial thickness as measured by ultrasonography in postmenopausal women is limited.[9, 10] So far, ultrasonography has been mainly used for evaluation of myometrial infiltration[11-15] in women with known endometrial cancer.

With the introduction of vaginal ultrasonography, ultrasonographic examination of the uterus in postmenopausal women has become very easy and quick. However, to the best of our knowledge, endovaginal ultrasonography has not been used for evaluation of endometrium in women with postmenopausal bleeding. This study was done to find out if there is any relation between endometrial thickness as measured by

From the Department of Obstetrics and Gynaecology, University of Göteborg, Sahlgrenska Hospital.
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Reprint requests: Seth Granberg, MD, Department of Obstetrics and Gynecology, Sahlgrenska Hospital, 413 45 Göteborg, Sweden.
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Fig. 1. Illustration of measuring endometrial thickness by endovaginal ultrasonography. *White arrows* indicate point where endometrium was measured in this patient. *Orientation:* Longitudinal scan with anterior position to right of figure and posterior position to left. Bottom of figure is toward vagina. *Size:* 1 cm between each *vertical line* at bottom of figure.

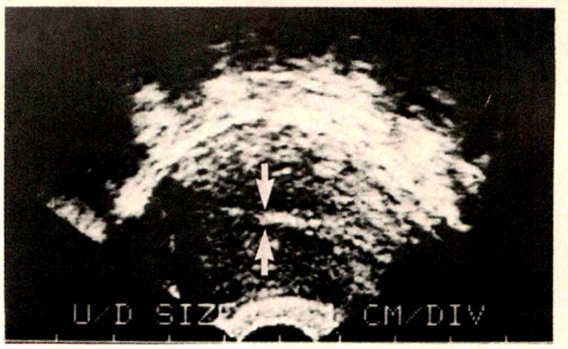


Fig. 2. Atrophic endometrium (between *white arrows*) as imaged by endovaginal scanning. Orientation and size as in Fig. 1.

Table I. Endometrial thickness as measured by vaginal sonography in women without postmenopausal bleeding and in woman with endometrial cancer diagnosed earlier

	No postmenopausal bleeding	*Cancer of endometrium*
Women (n)	30	30
Mean age (yr)	65.3	70.1
Thickness (mm, mean ± SD)	3.2 ± 0.7	17.7 ± 5.8
Range (mm)	2-5	9-34

vaginal ultrasonography and pathoanatomic diagnosis of the endometrium obtained at curettage in women with postmenopausal bleeding.

Material and methods

Two hundred five women with postmenopausal bleeding who were admitted to the hospital for curettage, were scanned endovaginally the day before operation. The ultrasonographic examinations were performed by use of a Brüel & Kjaer ultrasonography machine (model 1846) equipped with a vaginal transducer (model 1836) (Brüel & Kjaer, Naerum, Denmark). This mechanical transducer has a scanning sector of 112 degrees and a frequency of 7 MHz. The focal range of the transducer is 6 cm from its tip.

Before ultrasonographic examination, the patient emptied the urinary bladder. The examination was performed with the patient in lithotomy position. The transducer was introduced into the posterior vaginal fornix, and the uterus was scanned longitudinally and transversely. Endometrial thickness was measured at the thickest part in the longitudinal plane (Fig. 1). The measurement included both endometrial layers; that is, the measurement was performed between the two basal layers of the anterior and posterior uterine wall, also including the distended uterine cavity (Fig. 1). The poorly reflective layer surrounding the highly reflective endometrium was not included in the measurement, which was obtained by means of the electronic caliper built in the ultrasonography machine. All structures in the lower pelvis and urinary bladder that were considered abnormal by ultrasonography were noted. A curettage was performed by experienced gynecologists in the department.

The histopathologic diagnosis was used as the gold standard. In 11 patients the endometrial tissue obtained was insufficient for histologic diagnosis despite the fact that the surgeon stated that he or she had curetted the uterine cavity meticulously. According to principles used in our department, such an endometrium is classified as atrophic and benign even if it has not been possible to obtain a histologic diagnosis. For this reason these 11 patients were included in the study, and the endometrium was classified as atrophic and benign.

Within 2 weeks after curettage 15 patients were operated on because of a pelvic tumor found at ultrasonographic scanning before curettage. In all of these women hysterectomy and bilateral salpingo-oophorectomy were performed. Estrogen therapy

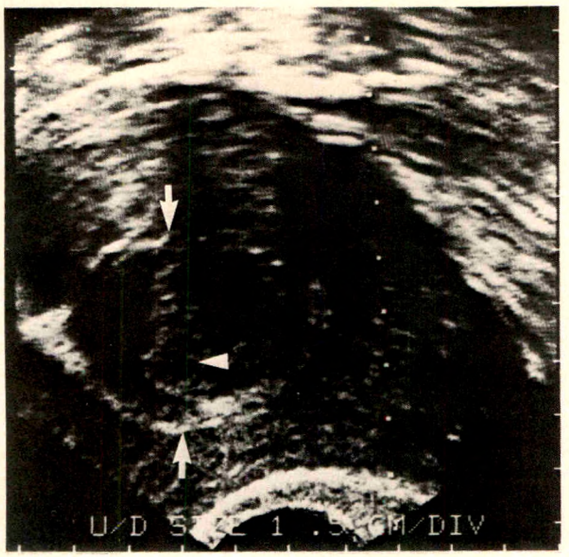


Fig. 3. Endometrial polyps *(white arrowhead)* as imaged by endovaginal scanning. *White arrows* indicate distended endometrial cavity. Orientation as in Fig. 1. *Size:* 1.5 cm between *vertical lines* at bottom of figure.

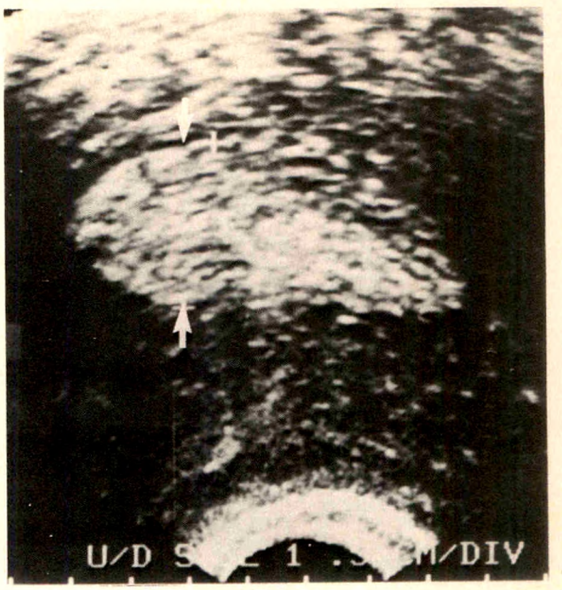


Fig. 4. Hyperplasia of endometrium (between *white arrows*) as imaged by endovaginal scanning. Orientation and size as in Fig. 3.

among the admitted patients was noted. Two groups of women were included as references. One group comprised 30 postmenopausal women without any vaginal bleeding admitted for surgical treatment of urine incontinence or prolapse. The other group comprised 30 women with previously diagnosed endometrial cancer. These 30 women underwent scanning 4 weeks after curettage, at which time the diagnosis was made but intracavitary radiation therapy had not been started. Five postmenopausal patients with vaginal bleeding were not included in the study since the initial gynecologic examination revealed a macroscopically visible tumor of the cervix.

Results

In 30 postmenopausal patients without vaginal bleeding, mean endometrial thickness was 3.2 ± 0.7 mm whereas the corresponding value for patients with known endometrial carcinoma was 17.7 ± 5.8 mm (Table I). Atrophic endometrium was found in 157 women (78%) (Fig. 2), endometrial polyps in 13 women (Fig. 3), endometrial hyperplasia in 13 (Fig. 4), and endometrial carcinoma in 8 women (9%) (Fig. 5) (Table II). Pyometra was found in three women, and one woman had hematometra (see Table II, "others"). In women with a histopathologic diagnosis of atrophic endometrium, mean endometrial thickness was 3.4 ± 1.2 mm (Table II). In women who had endometrial cancer, mean endometrial thickness as measured by vaginal

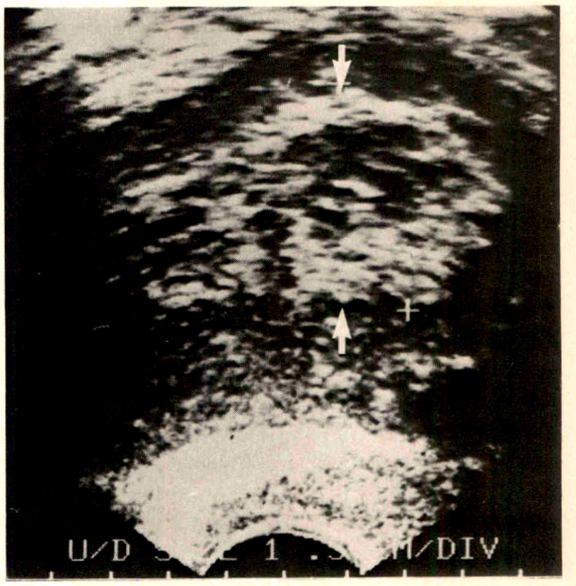


Fig. 5. Endometrial cancer as imaged by endovaginal ultrasonography. Note thick (between *white arrows*) and irregular outlining of endometrium. Orientation and size as in Fig. 3.

ultrasonography was 18.2 ± 6.2 mm (Table II). No endometrial cancer was found in endometrium that had a thickness of ≤8 mm. All endometrial carcinomas were found in patients with endometrial thickness of ≥9 mm (Table III).

Table II. Histologic diagnosis as related to endometrial thickness obtained by endovaginal scanning

	Final histologic diagnosis				
	Atrophy	*Polyp*	*Hyperplasia*	*Cancer*	*Other*
Women (n)	157*	13	13	18	4
Mean age (yr)	65.3	69.5	62.0	68.0	76.0
Thickness (mm, mean ± SD)	3.4 ± 1.2	10.8 ± 4.7	9.7 ± 2.5	18.2 ± 6.2	20.0 ± 7.9
Range (mm)	2-11	6-24	6-13	9-35	12-35

*Thirty of these women were on a regimen of estrogen therapy.

Table III. Histologic diagnosis of endometrium as related to thickness (5 mm intervals) of endometrium measured by endovaginal scanning

	Endometrial thickness						
Histologic diagnosis	*≤5 mm*	*6-10 mm*	*11-15 mm*	*16-20 mm*	*21-25 mm*	*>25 mm*	*Total No.*
Atrophy	150	6	1	—	—	—	157
Hyperplasia	—	7	6	—	—	—	13
Polyp	—	7	5	—	1	—	13
Pyometra	—	—	1	—	1	1	3
Hematometra	—	—	1	—	—	—	1
Cancer	—	1	8	4	3	2	18
TOTAL	150	21	22	4	5	3	205

Figures indicate number of women.

In all 30 patients who had been on a regimen of estrogen therapy at the time of postmenopausal bleeding, histologic diagnosis was atrophic (benign) endometrium. Five (17%) of these women had endometrial thickness of >5 mm (Table IV). Of those 127 women with benign histologic results who had not been receiving estrogen therapy, only two had endometrial thickness of >5 mm (Table IV). In this group of women the thickest endometrium was 7 mm, as compared with 11 mm in the group of women who had been on a regimen of estrogen therapy.

In 24 (11%) of the 205 women examined the ultrasonographic examination indicated the existence of other pelvic pathologic conditions that required further investigation. Final diagnosis in those women is shown in Table V. Fifteen of the 19 women who underwent laparotomy eventually underwent hysterectomy and bilateral salpingo-oophorectomy. In all but one woman the diagnosis obtained at curettage agreed with the histologic diagnosis of the endometrium obtained after surgery. This woman had an endometrial thickness of 9 mm but D & C revealed atrophy. The final diagnosis after laparotomy was a granulosa cell tumor and hyperplasia of the endometrium.

Comment

The diagnostic accuracy of curettage as compared with more simple procedures such as Vabra aspiration has been questioned.[1, 4] It has been shown by Stock and Kanbour[5] that in approximately 60% of the curettage procedures less than half of the uterine cavity is curetted. It must also be kept in mind that the overall costs for the vast number of curettages being performed are tremendous.[4] Furthermore, these elderly women have a small but real risk of morbidity and death.[4] With this in mind and the knowledge that only approximately 10% of women with postmenopausal bleeding have endometrial carcinoma, there is still a need to evaluate simpler, less expensive, and safer diagnostic methods that can exclude endometrial pathologic conditions. One such method is Vabra aspiration, which has been evaluated in several studies.[1, 4] Ultrasonography is another, fairly simple method that has been used for studying the endometrium.[7-9, 16] With high-frequency vaginal ultrasonography the endometrium can easily be visualized. Ultrasonography also has been used for studying endometrial carcinoma, but it has been difficult to find any specific echo that is characteristic of endometrial carcinoma.[11-15] On the other hand, ultrasonography has been shown to be a valuable instrument for staging known endometrial carcinoma.[11-15]

There are very few studies, however, where ultrasonography has been used for evaluation of endometrium in women with postmenopausal bleeding.[17, 18] Earlier studies within this field have used abdominal ultrasonography. Vaginal ultrasonography is a rather new technique that has not been extensively evaluated with regard to its use for early identification of endometrial abnormality in postmenopausal women.

The purpose of this study was to evaluate endome-

trial thickness as measured by vaginal ultrasonography as the only parameter for excluding endometrial abnormality. The mean endometrial thickness of 3.2 mm in postmenopausal women without any vaginal bleeding is similar to what has been found by other investigators by endovaginal ultrasonography.[17, 18] This figure is very similar to that (3.4 mm) found in women with vaginal bleeding but without any endometrial pathologic conditions as shown by curettage. In this study no endometrial abnormality was found if the endometrium was <6 mm thick. This is in agreement with Nasari and Coast,[17] as well as Fleischer et al.,[18] although they had fewer patients in their studies. It seems reasonable to have a cutoff limit for normal, "benign" postmenopausal endometrium at 5 mm (including both layers). In this study no endometrial cancer was found with endometrial thickness <9 mm, and mean endometrial thickness in those with endometrial carcinoma was 17.7 mm, which was almost the same as that for the 30 patients with previously diagnosed carcinoma of the endometrium (18.2 mm). The difference between thickness of atrophic endometrium, as measured by vaginal ultrasonography, and thickness of endometrium with carcinoma indicates that ultrasonography could be used as a very simple method to exclude endometrial carcinoma as the cause of postmenopausal bleeding. With regard to endometrial abnormality no false-negative observation was obtained if a 5 mm cutoff limit was used for endometrial thickness. This means a positive predictive value of 87.3%, a specificity of 96%, and a sensitivity of 100% for identifying endometrial abnormality with the presented technique. The corresponding figures for a cutoff limit of 4 mm were 60.8%, 80.2%, and 100%.

If these results hold true in the future, a vaginal ultrasonographic examination showing an endometrial thickness of <5 mm in a woman with postmenopausal bleeding could then mean that curettage could be avoided. It has been shown that approximately 80% of all curettages performed because of postmenopausal bleeding result in a benign diagnosis.[1, 3, 4, 6, 9] In this study 70% of curettages performed could have been avoided if the 5 mm limit had been used. Since a curettage is one of the most frequently performed gynecologic operations, a reduction of these by 50% would result in considerable savings[1, 4] with regard to both potential risks of the operation and economic costs.

It is interesting that endovaginal ultrasonographic examination in the 205 women with postmenopausal bleeding revealed other pelvic abnormality that would probably not have been diagnosed that early if the ultrasonographic examination had not been performed (Table V). It was unexpected to find five urinary bladder tumors. Of course it is not surprising that bleeding from the bladder can be mistaken for vaginal bleeding by the patient. The number of bladder tumors (5/205) is, however, probably too high since Benacerraf et al.[19] reported only three bladder tumors among 3000 gynecologic ultrasonographic examinations. The incidence of urinary bladder tumors in the age group 50 to 70 years is reported to be 28 per 100,000, which is much less than our figures would indicate.[20] However, one must remember that we have been studying a group of women with specific symptoms.

To evaluate the exact diagnostic accuracy of vaginal ultrasonography for excluding endometrial abnormaltiy, as well as other pelvic abnormality, as the cause of postmenopausal bleeding, one should include about 2000 to 3000 women in the study. To accomplish such an investigation within a reasonable time period, a multicenter study has been started in the Nordic countries. If the results presented here hold true, the number of curettages could be remarkably reduced, thereby benefiting the patients and saving money for the community.

Table IV. Endometrial thickness as measured by vaginal ultrasonography in women with and without estrogen therapy and a benign histologic diagnosis

Endometrial thickness (mm)	*No estrogen therapy*	*Estrogen therapy*	*Total*
2	24	1	25
3	65	8	73
4	26	14	40
5	9	3	12
6	1	1	2
7	1	3	4
11	—	1	1
Mean ± SD	3.2 ± 0.9	4.3 ± 1.7	3.4 ± 1.2
TOTAL	127	30	157

Table V. Final diagnosis in 24 women with ultrasonographic findings indicating pathologic structure in lower pelvis

Final diagnosis	*No. of women*
Simple ovarian cyst	5
Bladder tumor	5
Thecoma or fibroma	4
Ovarian cancer	3
Cystadenoma	2
Hydrosalpinx	2
Granulosa cell tumor	1
Hemorrhagic ovarian cyst	1
Cancer of colon	1
TOTAL	24

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The use of the Cytobrush cervical sampler in patients with cervical stenosis

Mitchel S. Hoffman, MD, Lorraine W. Gordy, CT, and Denis Cavanagh, MD
Tampa, Florida

In 37 of 43 patients (86%) with cervical stenosis (inability to undergo passage of the cotton-tipped applicator), the Cytobrush cervical sampler was successfully passed into the endocervical canal. The use of the Cytobrush sampler for cervical cytologic screening appears to be valuable in patients with cervical stenosis. (Am J Obstet Gynecol 1991;164:52-3.)

Key words: Cytology, cervical stenosis

The presence of endocervical cells is generally recognized as an important criterion for consideration of a cervical cytologic sample as adequate.[1] A variety of methods have been devised to improve endocervical cytologic sampling.[2] Several recent studies have suggested superior endocervical cell yield with the use of an endocervical brush (Cytobrush; M-Medscan AB, Malmo, Sweden; marketed in the United States by International Cytobrush, Inc., Hollywood, Fla.).[2] The present study was undertaken to evaluate the value of the Cytobrush sampler in patients with cervical stenosis.

Material and methods

Between Aug. 1, 1987, and Jan. 31, 1990, a separate "standard" Papanicolaou smear and an endocervical brush smear were obtained from each of the 491 consecutive patients entered into the study at the Tampa General Hospital and the H. Lee Moffitt Cancer Center and Research Institute colposcopy clinics. An analysis of the entire 491 patients will be the subject of another report.

From the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of South Florida College of Medicine at H. Lee Moffitt Cancer Center and Research Institute and Tampa General Hospital.
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Reprint requests: Mitchel S. Hoffman, MD, Division of Gynecology Oncology, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Dr., Tampa, FL 33612-9497.
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The first smear was obtained by using an Ayres spatula followed by a moistened cotton-tipped applicator in the endocervical canal. The moistened cotton-tipped applicator was inserted into the endocervical canal and rotated for 5 to 10 complete turns with slight pressure against the walls of the canal; an attempt was made to sample the entire endocervical canal. The material was immediately fixed on a standard glass slide with cytofixative. Immediately after this, a separate smear was obtained with the Cytobrush sampler by means of the same procedure that was used with the cotton-tipped applicator. Cervical stenosis was defined in this study as inability to introduce the moistened cotton-tipped applicator beyond the external os. In the event of cervical stenosis passage of the Cytobrush sampler into the endocervical canal was still attempted. Both smears were reviewed by cytotechnologists for the presence of endocervical cells. Patient data and results were collected prospectively during accrual. Patients were excluded from the study in the event of pregnancy or prior pelvic radiotherapy.

Results

Of the 491 total patients, 43 (7%) were identified as having cervical stenosis. The mean age of the entire patient population was 31 years (range 13 to 89 years). The mean age of the 43 patients was 42 years (range 26 to 89 years). Eighteen of the 43 patients were postmenopausal and 12 of the 43 patients had undergone prior cryotherapy. Two of the patients had undergone a laser conization of the cervix and two patients had undergone prior cold knife conization of the cervix. In 13 of the 43 patients (30%) endocervical cells were found on the "standard" Papanicolaou smear. In 38 patients (88%) the Cytobrush sampler was successfully passed into the endocervical canal. In one of these 38 patients the cytobrush specimen contained no endocervical cells, so that a total of 37 out of the 43 Cytobrush sampler specimens (86%) contained endocervical cells. With the χ^2 method for statistical comparison, the difference in endocervical cell yield between the "standard" Papanicolaou smear and the Cytobrush sampler was significantly different ($p < 0.01$).

Comment

As previously stated, the presence of endocervical cells is generally thought to be important for adequate cervical cytologic screening. Adequate sampling of the endocervical canal may be especially important in patients with cervical stenosis. The transformation zone in these patients is frequently within the endocervical canal, and in addition many of these patients have a history of treatment for precancerous lesions of the cervix. The very low endocervical cell yield (30%) by the "standard" Papanicolaou smear in patients with cervical stenosis in this study suggests that the standard Papanicolaou smear obtained with an Ayres spatula and a moistened cotton-tipped applicator is inadequate in these patients. We conclude from this study that the Cytobrush sampler is of value for obtaining an adequate cervical cytologic specimen in patients with cervical stenosis.

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Low level of maternal serum α-fetoprotein: Its associated anxiety and the effects of genetic counseling

Kathy L. Keenan, MS, Deborah Basso, MD, John Goldkrand, MD, and William J. Butler, MD

Albany, New York

A low maternal serum α-fetoprotein screening result can be expected to cause measurable anxiety for couples, particularly when the woman is younger than 35 years of age. This study was designed to investigate the levels of anxiety present in these patients and show the effectiveness of genetic counseling on this anxiety. The women and their partners were given the State Trait Anxiety Inventory before and after counseling. A control group of low-risk pregnant patients was also given the state anxiety inventory for comparison. The mean level of anxiety for the control group was 36, significantly lower than either the precounseling or postcounseling level of the group with low maternal serum α-fetoprotein screening results ($p < 0.05$). The study shows that genetic counseling, in addition to apprising couples of the risks and options involved, does significantly reduce their anxiety level regardless of whether an amniocentesis was performed. (AM J OBSTET GYNECOL 1991;164:54-6.)

Key words: Anxiety, low maternal serum α-fetoprotein, genetic counseling

As the national perinatal mortality rates progressively fall, congenital malformations and chromosomal abnormalities make an even larger contribution to fetal death and long-term disability. Chromosomal problems, principally Down syndrome, remain the leading cause of mental retardation. Despite the use of prenatal diagnosis, the estimated crude incidence of Down syndrome in the United States remains stable at 1 per 1000 births.[1] As the percentage of trisomy 21 births attributed to women over the age of 35 years declines, the number of Down syndrome births to women under the age of 35 reaches as high as 75% to 80%.[1]

In 1984, Merkatz et al.[1] reported that maternal serum α-fetoprotein levels were about 25% lower, on the average, in Down syndrome pregnancies than in unaffected pregnancies. This association has been repeatedly confirmed.[2, 3] Subsequently, Cuckle et al.[4] suggested that measurement of maternal serum α-fetoprotein might serve as a screening test for Down syndrome, even though a considerable overlap existed between the curves for affected and unaffected pregnancies. With maternal age of 35 years as the lone criterion for offering prenatal diagnosis, approximately 20% of all Down syndrome cases would be detected. The finding that low levels of maternal serum α-fetoprotein were associated with Down syndrome pregnancies meant that now it would be possible to justify offering amniocentesis to the at-risk younger population to detect an additional 20% of Down syndrome pregnancies.

The mental anguish and apprehension that a woman encounters when she is told she is at risk of having a baby with Down syndrome on the basis of a low level of maternal serum α-fetoprotein have been addressed in the literature.[3] The knowledge of this risk plus the thought of having to undergo an invasive procedure (amniocentesis) have been shown to create far more anxiety in the younger woman with a low level of maternal serum α-fetoprotein than in a woman age 35 years and older.[5] Although the anxiety of patients before counseling or prenatal diagnostic testing has been quantified by Evans et al.,[5] the effect of counseling for either alleviation or exacerbation of anxiety has not been previously tested.

Methods

The State Trait Anxiety Inventory designed by Spielberger and colleagues[6] provides a means to measure transitory anxiety levels (i.e., anxiety levels that fluctuate because of external circumstances). The scores obtained on the state anxiety inventory can range from 20 to 80. The greater the level of patient anxiety the higher the score. A slight variation in score indicates large differences in the level of anxiety. The manual that accompanies the test and serves as a guide to score and interpret the state anxiety inventory gives a few examples of anxiety levels. The mean anxiety state of the average medical patient is 42 and the average patient with acute anxiety reaction is 48.

From the Department of Obstetrics and Gynecology, Albany Medical Center.
Received for publication May 30, 1990; revised August 27, 1990; accepted August 31, 1990.
Reprint requests: Kathy L. Keenan, Department of Obstetrics and Gynecology, A-166, Albany Medical Center, Albany, NY 12208.
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Private obstetricians or obstetric clinics referred patients to Albany Medical Center after one or sometimes two test results showed low levels of maternal serum α-fetoprotein. Each patient first met with the genetic counselor. After counseling an ultrasonographic evaluation was performed to confirm gestational age, with the option of an amniocentesis if indicated and desired by the patient.

Between Jan. 1 and Oct. 31, 1988, all patients (couples) who were referred for genetic counseling with regard to low levels of maternal serum α-fetoprotein were asked to participate in this study. Two eligible patients refused. Fifty-two patients agreed to take the state anxiety inventory before and after the counseling session. Each patient (couple) met with the counselor for approximately 45 minutes. This time was spent reviewing pertinent information concerning pregnancy and family histories, imparting risk data, and addressing patient questions and concerns. The counselor explained that a low level of maternal serum α-fetoprotein provides an adjustment of a woman's age-related risk of Down syndrome. Patients were also counseled with regard to the impact of gestational age on evaluation of maternal serum α-fetoprotein. Ultrasonographic and amniocentesis procedures were reviewed including the 1 in 250 miscarriage risk associated with amniocentesis.

Our control group consisted of 25 women from the same private obstetric practices as our study group. These women had normal maternal serum α-fetoprotein screening results and were currently being followed up by their obstetricians as low-risk patients. These women were all younger than 35 years of age and from similar socioeconomic, geographic, and educational backgrounds as the women in our study group. Each control patient agreed to participate and complete the state anxiety inventory.

The data were statistically analyzed with the Student *t* test and Mann-Whitney U test.

Results

Our results are summarized in Table I. In our study group, the mean anxiety score when study participants were first seen for counseling was 52. After counseling the mean score dropped to 47 ($p < 0.05$). It is interesting to note that the women initially had much higher anxiety scores than those of their partners (56.5 vs 44.5) and that the women seemed to benefit more from counseling than did the men as evidenced by a greater drop in the anxiety level of the women after counseling (7.0 vs 1.5). The women, when analyzed separately, had a very significant change in anxiety level ($p < 0.01$). However, the men did not experience a significant decrease in anxiety level.

Fig. 1 illustrates the mean anxiety level in the control group versus that in the precounseling and postcounseling groups. The mean anxiety level in our control group was significantly lower than the mean anxiety level in either the precounseling or postcounseling group with low maternal serum α-fetoprotein screening results. It was evident that in general there is elevated anxiety reaction for the patient informed of her increased risk of having a baby with Down syndrome. The control versus precounseling anxiety level is 36 vs 52 ($p < 0.05$). Although the increased anxiety level may persist at least until a definite chromosomal diagnosis by amniocentesis, this initial anxiety can be reduced through comprehensive genetic counseling.

Table I. Summary of results

	Mean anxiety levels			
	Precounseling	*Postcounseling*	*Change*	*p Value*
Participants	52	47	−5	<0.05
Women alone	56.5	49.5	−7	<0.01
Men alone	44.5	43.5	−1.5	>0.05*

*Not significant.

Comment

The maternal serum α-fetoprotein screening test can be a source of patient concern and apprehension. A screening test, independent of maternal age, that would help to identify a subset of pregnancies at risk of an aneuploidy would be of enormous value. The maternal serum α-fetoprotein screening test is not ideal for pregnancies already at risk of an autosomal trisomy.[2] At the maternal serum α-fetoprotein level commonly used for identification of the pregnancies at risk (i.e., calculated risk at 1:270, which correlates with the risk at age 35 years) the sensitivity is approximately 30%. The specificity is also less than ideal. Low levels of maternal serum α-fetoprotein are frequently a result of many factors independent of fetal aneuploidy (incorrect dates, fetal death, choriocarcinoma, elevated maternal weight).

A source of referral for information and guidance should be available to the patient once a low maternal serum α-fetoprotein result is reported. With the use of the state anxiety inventory, we quantified the level of anxiety a low maternal serum α-fetoprotein test result may induce in a patient and the effect that comprehensive genetic counseling can have on this level of anxiety. Counseling reduced the anxiety of these patients. These results contrast with previously published data by Burton[7] in which she investigated anxiety produced by elevated levels of maternal serum α-fetoprotein. She concluded that heightened anxiety was not alleviated by counseling before definitive testing.

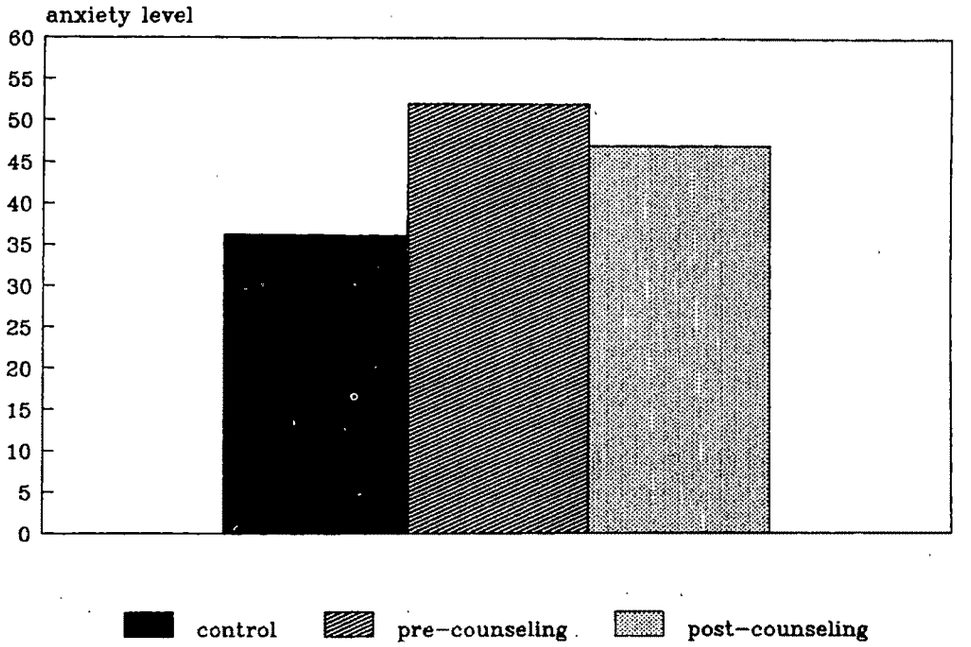


Fig. 1. Mean anxiety.

Although our patients' anxieties were not lowered to levels equivalent to the control group, counseling did significantly reduce their anxiety level. The State Trait Anxiety Inventory was used by both Burton's study and our study. The patients and their partners in our study had an average precounseling anxiety level of 52. In the study by Burton the average anxiety level was approximately 47 and did not change after counseling. In our study, the average anxiety level after counseling dropped to 47. It is possible that our finding of a significant decrease in anxiety level after counseling is attributable to the higher baseline anxiety rate noted in our patients. This higher anxiety level may reflect the patients' perception of the seriousness of the fetal abnormality for which their pregnancy is at increased risk. No data are available to compare perceived risks of neural tube defects with fetal aneuploidy.

In those cases in which amniocentesis reveals a trisomic fetus, it may be said that the cost of the many false-positives was justified. For those pregnancies that are identified, the parents have various options including termination of the pregnancy, exploration of medical and surgical management decisions before delivery, arrangement of an adequate support systems, or just time to cope with the unfulfilled expectation of having a healthy baby. For now, maternal serum α-fetoprotein as a screen for Down syndrome and other trisomies continues to be a controversial issue.

A lower false-positive rate would greatly increase the utility of the screening test and reduce the number of unnecessarily anxious patients. Tests that look at other risk parameters may be integrated into screening programs to improve the sensitivity and specificity of the aneuploidy risk.

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Successful antepartum treatment of listeriosis

Charles Kalstone, MD
Miami, Florida

A pregnant patient had a flulike illness at 27 weeks. *Listeria monocytogenes* infection was diagnosed by blood cultures. Electronic monitoring suggested the fetus was stressed. Use of tocolytics inhibited uterine contractions while the mother was treated with intravenous ampicillin. Four days later when labor began because of chorioamnionitis, the infant was delivered in good condition. (AM J OBSTET GYNECOL 1991;164:57-8.)

Key words: *Listeria monocytogenes,* blood cultures, sporadic or epidemic

A case of maternal and fetal *Listeria monocytogenes* infection is reported in which antepartum recognition, uterine tocolysis, antibiotic therapy, and preterm delivery led to a successful outcome.

Case report

A 26-year-old primigravid woman had a normal antepartum course until 27 weeks' gestation when a flulike illness developed with fever, backache, and myalgias. Results of routine urinalysis were normal. After 1 week a blood-tinged, nonpurulent vaginal discharge developed. The membranes were intact, and ultrasonographic examination showed a normal 28-week fetus and placenta. The cervix was not dilated or effaced. The patient was not aware of uterine contractions but external monitoring demonstrated them.

The patient was admitted to the labor room. Except for a temperature of 100.5° F, the physical examination was normal. There were no localizing signs; white blood count was 10,300/mm³ with a normal differential, and hemoglobin was 9.7 gm. Two blood cultures were drawn and vaginal and cervical cultures were taken.

The fetus was active and external monitoring showed a normal fetal heart rate with a reactive pattern. Uterine contractions were mild and occurred every few minutes; administration of two subcutaneous doses of terbutaline, 0.25 mg, 20 minutes apart, stopped them. The cervix remained long and closed. Terbutaline injections were continued every few hours; after 12 hours the drug was given orally at a dosage of 5 mg every 4 hours.

The patient's fever continued and reached 102° F. The bloody vaginal secretions also continued. Two days later the mother reported a lack of fetal movement. External fetal monitoring showed a nonreactive pattern that continued for 12 hours; there were two uterine contractions, each associated with a late deceleration.

Vaginal and cervical cultures were negative but both blood cultures taken on admission were growing *L. monocytogenes.* An infectious disease consultant suggested the use of intravenous ampicillin, which was administered at a dosage of 3 gm every 4 hours. Oral terbutaline was continued, and within 24 hours fetal activity returned to normal.

The maternal fever, flulike symptoms, and bloody discharge from the uterus continued. Three days later labor began and there was active bleeding. The cervix was undilated but effaced. The presentation was vertex. External fetal monitoring demonstrated a reactive fetal heart pattern with no late decelerations despite uterine contractions every 3 minutes. Because the mother was still ill, no further tocolysis was indicated. It was deemed best to deliver the infant promptly, while it had recovered from the intrauterine stress.

A low vertical cesarean section was performed with the mother under epidural anesthesia. The placenta was necrotic; microscopically it contained microabscesses, villositis, acute chorioamnionitis, and umbilical angiitis. Culture of the membranes revealed light growth of *L. monocytogenes.*

The infant, a 1100 gm male, had Apgar scores of 6 at 1 minute and 8 at 5 minutes. He was intubated in the delivery room, transferred to the neonatal intensive care unit, and received mechanical ventilation and oxygen for moderate respiratory distress syndrome. His white blood cell count was 32,000/mm³ with 45 bands, consistent with intrauterine infection. He was treated with ampicillin and gentamicin. On day 3 his white blood cell count was 70,000/mm³ with 30% monocytes. Cultures of his blood, gastric and tracheal aspirates, and spinal fluid were negative. When he was extubated on day 6, he became acutely hypoxic as a result of a pulmonary hemorrhage. He was immediately reintubated and there was no further pulmonary bleeding. Ultrasonography showed no intracranial hemorrhage. Five days later he was reextubated and did well. Intravenous ampicillin was given for 14 days. He was discharged in excellent condition on day 50, weighing 1940 gm.

The mother's illness resolved with delivery. She was discharged in 1 week and was treated with intravenous ampicillin for an additional 2 weeks.

From the Department of Obstetrics and Gynecology, South Miami Hospital.
Received for publication May 18, 1990; accepted July 5, 1990.
Reprint requests: Charles Kalstone, MD, 6280 Sunset Dr., Suite 500, South Miami, FL 33143.
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Comment

Listeria infections occur primarily in pregnant women, newborns, elderly, and immunocompromised persons. A flulike illness that persists in a pregnant patient, especially in the third trimester, can be caused by *L. monocytogenes* infection. Cervical and vaginal cultures often show no growth of this organism. For this reason, blood cultures are the best means to establish the diagnosis. The infection is bloodborne and spreads to the fetoplacental unit.

Untreated listeriosis has a high perinatal morbidity and mortality. Prompt maternal antibiotic therapy can save the fetus in utero. Intravenous ampicillin is the treatment of choice; it achieves desirable drug levels in the fetus. In penicillin-allergic patients, use of sulfamethoxazole and trimethoprim has been successful.

Cases of listeriosis are sporadic or epidemic. When sporadic, the source is usually not determined. Our patient ate imported Gouda cheese around the time that she became ill but none was left to test for listeria infection. There have been reports of epidemic outbreaks of listeriosis acquired from eating cheese made from unpasteurized milk.[1] Pregnant patients should be warned not to eat cheese made from unpasteurized milk. Improper curing, another alternative in cheesemaking, may not kill the listeria organism.

Others have reported successful antibiotic treatment of maternal listeria septicemia in the second trimester with resultant delivery of a normal infant at term.[2] The unique aspects of this case report are that tocolytics were used to inhibit preterm uterine contractions while maternal antibiotic therapy reversed the fetal stress demonstrated by external fetal heart monitoring. However, administration of antibiotics did not cure the mother. Fever persisted, as did bloody discharge from the uterus, an important sign that is probably caused by placental necrosis from intrauterine infection. This can cause labor by release of prostaglandins from the decidua and membranes.

In cases like this one, when it may not be possible to continue to inhibit preterm labor by tocolytics, initiating maternal antibiotic therapy may control fetal infection until the infant is born. Antibiotic therapy then can be continued in the neonate. Although uncommon, listeriosis is a preventable cause of perinatal mortality.

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Anemia and spontaneous preterm birth

Mark A. Klebanoff, MD, MPH,[a] Patricia H. Shiono, PhD,[a, b] Joseph V. Selby, MD, MPH,[c] Alan I. Trachtenberg, MD, MPH,[d] and Barry I. Graubard, MA[e]

Bethesda, Maryland, and Oakland, California

The association between anemia during pregnancy and spontaneous preterm birth was studied with a two-stage case-control design in a large, multiethnic cohort. Results of all hematologic measurements were abstracted from the prenatal and delivery records of 1706 of the 26,901 women in the cohort. Among women delivered of infants at term, mean hematocrit value was low during the early phase of the second trimester, stable until near term, then reached a maximum at 40 weeks' gestation. The mean hematocrit value of black women was consistently lower than that of Asian, Mexican, and white women. Anemia (hematocrit value less than the tenth percentile for ethnic group and duration of pregnancy) at any time during the second trimester was positively associated with subsequent spontaneous preterm birth (odds ratio, 1.9; 95% confidence interval, 1.3 to 2.8). Compared with white women, the odds ratios for preterm birth were 2.0 (95% confidence interval, 1.6 to 2.4) for black, 1.2 (95% confidence interval, 0.9 to 1.6) for Asian, and 1.2 (95% confidence interval, 1.0 to 1.5) for Mexican women. Adjustment for second-trimester anemia had minimal influence on the odds ratios. We conclude that anemia during the second trimester was associated with preterm birth. However, it does not account for the large ethnic differences in preterm birth. (AM J OBSTET GYNECOL 1991;164:59-63.)

Key words: Anemia, hematocrit, pregnancy, premature labor

Anemia, as measured at the time of delivery, has been associated with spontaneous preterm birth.[1, 2] The study reported that a hematocrit value of 34 or less was strongly associated with preterm birth and that there was a dose-response relationship between hematocrit value and preterm birth. Anemia was found to account for 60% of the observed differences between black and white women in the occurrence of spontaneous preterm birth.[1] These findings were later questioned in a subsequent study[3] that showed that because the hematocrit value rises during the third trimester of a normal pregnancy, comparison of delivery hematocrit values in women giving birth to term and preterm infants produces a spurious association between anemia and preterm birth. The purpose of the present study is to explore further the association between hematocrit measured at various gestational ages before delivery and duration of pregnancy. To accomplish this, a case-control study[4, 5] was carried out within a large, multiethnic pregnancy cohort.

From the Division of Prevention Research, National Institute of Child Health and Human Development,[a] the Center for the Future of Children, David and Lucile Packard Foundation,[b] the Permanente Medical Group, Division of Research,[c] Bay Area Addiction Research and Treatment,[d] and the Biometry Branch, Division of Cancer Prevention and Control, National Cancer Institute.[e]

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Reprint requests: Mark A. Klebanoff, MD, Division of Prevention Research, National Institute of Child Health and Human Development, National Institutes of Health, EPN 640, Bethesda, MD 20892.

6/1/24741

Methods

The data for this study are from the northern California Kaiser Permanente Birth Defects Study. Detailed methods of this prospective cohort study are published elsewhere.[6] Women who registered for prenatal care at the 13 facilities that served northern California during 1974 to 1977 completed a self-administered questionnaire in English or Spanish that obtained information on a variety of demographic, reproductive, and behavioral variables. Gestational age was determined on the basis of the menstrual history and physical examination at the first prenatal visit. Preterm delivery was defined as a live birth of 24 to 36 completed weeks' gestation. There were 36,504 women initially recruited; exclusion of women found not to be pregnant, women who had induced abortions, women who wanted the infant to be adopted, women not delivered of infants at a Kaiser hospital, and women lost to follow-up resulted in outcome data for 34,660 babies. Women delivered of a live-born singleton infant of at least 24 weeks' gestation and 500 gm were included in these analyses (n = 31,682). Repeat study pregnancies by the same women were excluded (n = 1086), as were women who started prenatal care after 24 weeks' gestation (n = 2266) and women of unknown race (n = 1429). The final sampling frame contained 26,901 women.

In the original study, data on hematocrit values and whether delivery was induced were not collected. So

Table I. Derivation of total number of births

	Asian	*Black*	*Mexican*	*White*	*Total*
Preterm					
Full cohort	152	306	243	1194	1895
Sampled	152	306	243	401	1102
Known induction or no labor	17	55	35	57	164
Labor status unknown	41	53	49	70	213
Known spontaneous preterm labor and 1 or more hematocrit values present (cases)*	94	198	159	274	725
Estimated number of spontaneous preterm births in full cohort*	129†	239†	199†	988†	1555
Term					
Full cohort*	1771	2228	2538	18469	25006
Sampled	152	306	243	401	1102
One or more hematocrit values present (controls)*	140	279	200	362	981

*Values were used to adjust the odds ratios for ethnicity and preterm birth and the adjustments to their variances.

†Derived by multiplying the quotient of the number of women with known spontaneous preterm labor with one or more hematocrit values (a), divided by the sum of the number of women with known spontaneous preterm labor with one or more hematocrit values (a) and the number of women with known induction or no labor (b), times the number of women in the full cohort (c): $[a/(a + b)] \times c$.

that these data could be gathered by review of the records of a manageable number of preterm and term pregnancies, a two-stage case-control design was used.[4, 5] All preterm infants born to Asian (n = 152), black (n = 306), and Mexican (n = 243) women were selected, as was a simple random sample of 401 of the 1194 preterm infants born to white women. Within each ethnic group, one control woman delivered of an infant after 36 weeks' gestation was selected at random for each case. Hematocrit data were abstracted from both the inpatient and outpatient records; data on induction of labor were abstracted from the inpatient records.

Of the 2204 women in the sample, charts for 334 were not available, primarily because of off-site storage and personnel shortages at certain facilities. Therefore women with missing charts are unlikely to differ from the remainder of the sample. Inasmuch as spontaneous preterm birth was the outcome of interest, preterm infants born after induced labor or cesarean section in the absence of labor were excluded (n = 164), which reduced the number of records to 1706. The final sample analyzed consisted of women who had spontaneous preterm labor and one or more hematocrit values (n = 94, Asian; 198, black; 159, Mexican; 274, white), or term delivery and one or more hematocrit values (n = 140, Asian; 279, black; 200, Mexican; 362, white). Details of the sampling procedure are shown in Table I.

All measures of hematocrit or hemoglobin and the date they were obtained were abstracted from the records. Data on the source of blood (venous or finger stick), the method used to calculate hematocrit (centrifuged or Coulter counter) and the cause of anemia (iron deficiency or other) were not obtained. The gestational age at each measurement was determined by subtracting the date of the last menstrual period or physician's estimate of the last menstrual period from the date the blood was obtained. On average, each woman had 2.6 measures of hematologic status; 12% had one measure, 31% had two, 36% had three, 17% had four, and 4% had five or more. In those instances in which only a hemoglobin value was available, it was converted to an equivalent hematocrit value by multiplying by three.

Our definition of anemia varied slightly depending on the specific analysis. When the relationship between anemia and subsequent preterm birth was studied, ethnic group-specific definitions of anemia were used to separate the effect of anemia from that of ethnicity. Thus for each ethnic group anemia was defined as a hematocrit value of less than the tenth percentile for women of that ethnic group during each 2-week interval of gestational age on the basis of the measurements of pregnancies that ultimately ended at term. The distribution of hematocrit values was similar for all nonblack women; therefore these groups were combined. The distribution of hematocrit values among black women was shifted toward lower values. Dependent on the specific interval, the tenth percentile of hematocrit was 31 to 33 for nonblack and 29 to 31 for black women.

When the role of anemia in explaining the ethnic differences in preterm birth was studied, anemia was defined as the tenth percentile for all term infants in the sample, weighted for the unequal sampling probabilities of each ethnic group. If different tenth percentiles were used for each ethnic group, the prevalence of anemia would not differ between ethnic groups and adjustment for anemia could not effect the relationship between ethnicity and preterm birth.

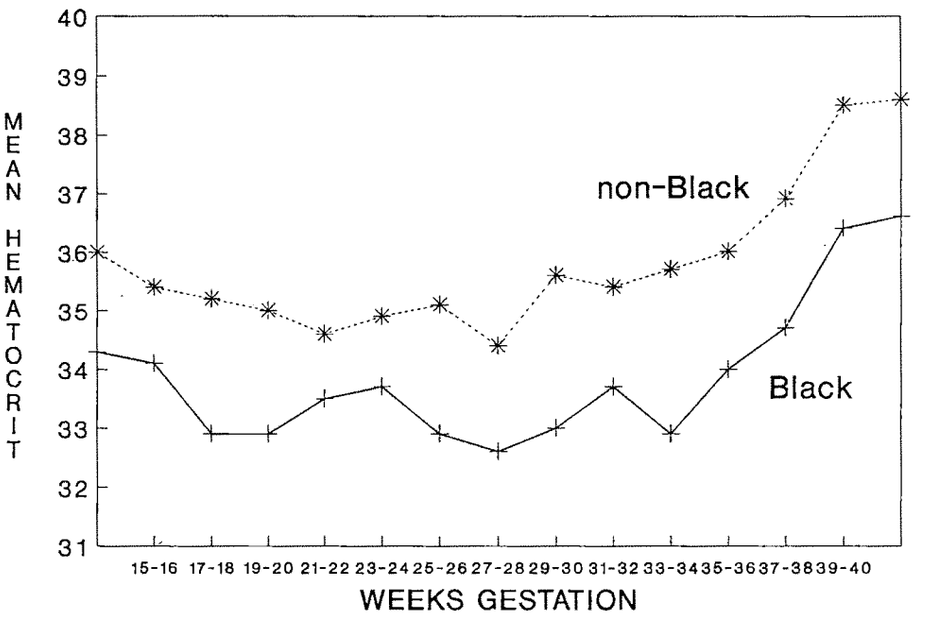


Fig. 1. Mean hematocrit value at each 2-week interval of gestation among pregnancies delivered at term.

Statistical methods. As described, all Asian, black, and Mexican preterm infants were studied, as were approximately one third of the white preterm infants. Term infants were selected as controls from each ethnic group according to the number of preterm cases from that group. Because the sampling probabilities were known and differed by outcome and ethnic group, odds ratios for ethnicity could be calculated by adjusting for the different sampling fractions according to the method described by Cain and Breslow.[5] This method adjusts both the odds ratios and the SEs derived from logistic regression for unequal sampling probabilities.

To compare our results with those previously described,[3] the change in hematocrit value during a normal pregnancy that resulted in a term infant was evaluated. The mean hematocrit value at each 2-week interval during the second and third trimesters for women delivered of infants of >36 weeks' gestational age was studied. Next, we evaluated the association between anemia as determined at each 2-week interval of gestational age and subsequent preterm birth. In addition, the occurrence of preterm birth was compared between women who were anemic at any time during the second trimester and women who had hematocrits measured during the second trimester and were not anemic. This analysis was done for all women and separately for women in each ethnic group. Finally, we evaluated the role of anemia in explaining the ethnic differences in preterm birth. The odds ratios were calculated for preterm birth to Asian, black, and Mexican women compared with white women. The contribution of differing rates of anemia to the ethnic differences in preterm birth was assessed by comparison of the odds ratios before and after adjustment for the presence of anemia at any time during the second trimester. Logistic regression was used for these analyses.

Results

The mean hematocrit value at each 2-week interval of gestation during the second and third trimesters is presented in Fig. 1 for black and nonblack women delivered of infants at term. Hematocrit values appeared to fall during the early second trimester, reach a nadir at approximately 26 to 28 weeks' gestation, begin to increase again at 31 to 33 weeks, then increase sharply at term, reaching a maximum at 40 weeks' gestation. Throughout the second and third trimesters, hematocrit values were approximately 2% lower among black than among nonblack women.

Because hematocrit values varied widely by gestational age, the relationship between preterm delivery and anemia was evaluated at 2-week intervals of gestational age (Table II). Odds ratios for preterm birth and anemia were adjusted for ethnicity, age, education, marital status, smoking, and gestational age at first prenatal visit. The odds ratios for anemia during the period from 13 to 26 weeks' gestation were >1.0, indicating an increased risk of preterm delivery among anemic women. However, anemia after 26 weeks' gestation generally had an inverse relationship with preterm birth. Having been anemic at any time during the second trimester (weeks 13 to 26) was positively asso-

Table II. Odds ratios for preterm delivery and anemia by gestational age

Weeks' gestation	*No.*	*Adjusted odds ratio**	*95% CI*
13-14	284	1.4	0.5-3.7
15-16	199	2.1	0.6-7.4
17-18	148	4.3	1.2-15.5†
19-20	163	1.4	0.4-5.4
21-22	197	2.0	0.8-4.9
23-24	203	1.4	0.5-3.7
25-26	163	3.5	1.1-11.3†
27-28	218	0.8	0.3-2.1
29-30	267	1.4	0.6-3.3
31-32	408	0.5	0.1-1.5
33-34	328	0.7	0.3-1.6
35-36	391	0.5	0.2-1.3

*Adjusted for ethnicity, maternal age, education, marital status, smoking, gestational age at first prenatal visit.
†$p < 0.05$.

ciated with preterm birth [odds ratio, 1.9; 95% confidence interval (CI) 1.2 to 3.0]. The odds ratios by ethnic group were 2.6 (95% CI, 0.7 to 9.6) for Asian women, 1.8 (95% CI, 0.9 to 3.6) for black women, 2.0 (95% CI, 0.8 to 4.7) for Mexican women, and 1.8 (95% CI, 1.0 to 3.5) for white women. Anemia at any time during the third trimester was not analyzed because women delivered of infants at term had more opportunity than did women delivered of infants before term to have anemia diagnosed. This does not apply to the biweekly analyses in Table II because only women who were still pregnant during that interval could have their hematocrit measured.

The role of anemia in the explanation of the ethnic differences in preterm birth is shown in Table III. The unadjusted odds ratios for preterm delivery among Asian, black, and Mexican women, compared with white women were 1.2, 2.0, and 1.2, respectively. Despite the association between second-trimester anemia and preterm birth, adjustment for the presence of anemia at any time during the second trimester had little effect on the ethnic differences in preterm delivery. Anemia after 26 weeks' gestation was negatively associated with preterm birth; therefore it could not possibly account for the observed ethnic differences.

Comment

We found that in pregnancies carried to term, the mean hematocrit value rises dramatically after 37 weeks' gestation. This is in agreement with earlier reports,[3] and with the standards developed by the Centers for Disease Control for hematocrit values during pregnancy.[7] Because the same group of women contributed data over most of pregnancy, our study has advantages over purely cross-sectional data in which entirely different women may have had blood drawn during each interval. However, our data are not fully longitudinal; every woman did not have an hematocrit drawn during every interval. To the extent that women who had blood drawn during any given interval differed from those who did not, our results will be in error. Our results agree closely with established standards,[7] and are consistent with the observation that plasma volume is relatively constant after 34 weeks' gestation but red cell mass increases linearly throughout pregnancy.[8] This suggests that the lack of regular measurements taken longitudinally on each woman was unlikely to influence the results.

Our results confirm those of Klebanoff et al.,[3] who found that when measured prospectively, third-trimester anemia was not a risk factor for preterm birth. Our results contradict those of Lieberman et al.,[1] who found a very strong dose-response relationship between preterm birth and third-trimester anemia (hematocrit value ≤34) and that anemia accounted for much of the difference in preterm delivery rates between black and white women. Lieberman et al.[1] defined anemia with hematocrit values obtained at delivery. Our data suggest that the use of delivery hematocrit values is inappropriate because hematocrit rises sharply at term. Use of delivery hematocrit values will therefore produce a spurious relationship between anemia and preterm birth.

We found a moderate relationship between anemia during the second trimester and preterm birth. Anemia during the third trimester tended to be negatively associated with preterm birth. In addition, we found that statistical adjustment for the presence of anemia did not affect the ethnic differences in preterm delivery previously reported in this cohort.[9] The association between second-trimester anemia and preterm delivery is not inconsistent with previous findings on this subject because prior work did not evaluate anemia during the second trimester.[3]

Why should second-trimester anemia be associated with preterm delivery when anemia later in pregnancy is not? There is no clear answer. This association has not been reported previously, and it may be an artifact of multiple comparisons. The relationship was consistent within ethnicity, making simple chance less likely. It is possible that a small group of high-risk, severely anemic women had hematocrits measured on multiple occasions. This detection bias would tend to overstate the relationship between preterm birth and anemia. We found that women who had one hematocrit measured during the second trimester were no more likely to be delivered of preterm infants than were women who had none. However, women who had two or more hematocrit measurements during the second trimester were at elevated risk of preterm delivery. In an attempt to reduce detection bias, we analyzed mean and first hematocrit values during the second trimester. The results were unchanged when anemia was defined with the use of the mean of all hematocrit values obtained during the second trimester and with the first hemat-

Table III. Odds ratios and 95% CI for preterm delivery by ethnic group

	White	*Asian*		*Black*		*Mexican*	
	Odds ratio	*Odds ratio*	*95% CI*	*Odds ratio*	*95% CI*	*Odds ratio*	*95% CI*
Unadjusted	1.0	1.2	0.9-1.6	2.0	1.6-2.4*	1.2	1.0-1.5*
Adjusted†	1.0	1.2	0.9-1.6	1.9	1.5-2.3*	1.2	1.0-1.6

*$p < 0.05$.
†Odds ratio adjusted for the presence of second-trimester anemia.

ocrit value obtained during the second trimester. Nevertheless, the association between anemia during the second trimester preterm birth should be considered tentative and needs to be confirmed in other populations.

A precise definition of anemia during pregnancy is confounded by various factors such as altitude, ethnicity, use of iron supplements, and changes in plasma volume during pregnancy. Definitions of anemia have ranged from hematocrit values of ≤34,[1] to a hemoglobin of <10 gm per deciliter[10] (corresponding to hematocrit value of 30), to the Centers for Disease Control's month-specific standards.[7] Use of either of the two fixed values (≤34 or <30) produced similar results in our data. Because there is no standard definition of anemia for 2-week intervals during pregnancy, we chose to use the internal standard of the tenth percentile at each 2-week interval, as determined among pregnancies that ultimately resulted in births at term. When evaluating the role of anemia, separate standards were used for black and nonblack women to eliminate ethnicity as a confounder. When evaluating the role of ethnicity in preterm birth, a single standard for anemia was used so as not to overadjust for ethnicity.

Rapid changes in red cell mass and plasma volume during pregnancy make interpretation of isolated hematocrit values difficult. Low hematocrit levels may result from inadequate red cell mass or a large expansion of plasma volume. Inadequate expansion of plasma volume, which would result in high hematocrit values, has been associated with an increased risk of a variety of pregnancy complications.[11, 12] Women with normal red blood cell mass and a large expansion of plasma volume would probably be at low risk of adverse outcome despite an apparent anemia. Therefore further studies with clinical determinations of hematocrit value are unlikely to resolve this issue. Anemia in pregnancy should be defined as a function of red cell mass and plasma volume, neither of which are measured in clinical settings. In addition, measured hematocrit is a result of the interaction of physiologic, nutritional, and other life style factors. Despite any observed associations, low hematocrit values may merely be a marker for social and environmental deprivation, rather than being a cause of preterm birth. Therefore treatment of anemia alone might not affect the occurrence of spontaneous preterm birth.

In conclusion, anemia during the second trimester was found to be positively associated with spontaneous preterm birth; anemia later in pregnancy tended to be negatively associated with preterm birth. Second-trimester anemia did not contribute to the observed ethnic differences in the occurrence of preterm birth. Future studies of anemia during pregnancy should supplement routine clinical data with detailed measures of red cell mass and plasma volume. These additional measurements may clarify the role of anemia in the pathogenesis of preterm birth as well as explain the observed ethnic differences in hematocrit values.

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Fetal urine output does not influence residual amniotic fluid volume after premature rupture of membranes

William J. Watson, MD, Vern L. Katz, MD, and John W. Seeds, MD
Chapel Hill, North Carolina

Fetal urine production in 12 women with premature rupture of membranes did not differ from that of a control group, and there was no correlation between fetal urine output and residual amniotic fluid volume. Residual amniotic fluid volume after premature rupture of membranes does not appear to depend on or alter fetal renal function. (AM J OBSTET GYNECOL 1991;164:64-5.)

Key words: Premature rupture of membranes, amniotic fluid, fetal urination

Pregnancies complicated by preterm premature rupture of membranes are at increased risk for adverse outcome if the residual amniotic fluid volume is low. Urine production may also depend on fetal oral intake, which may be reduced with low amniotic fluid volumes. We hypothesized that residual amniotic fluid volume in pregnancies complicated by premature rupture of membranes may be related to fetal urine production.

Methods

This study was approved by the institutional committee on research of human subjects. Fetal urine output was estimated ultrasonographically by the method of Campbell et al.[1] in 12 gravid women with premature rupture of membranes at 26 to 34 weeks' gestation and compared with similar estimates in a control group of 12 women with uncomplicated pregnancies of similar gestational ages and with intact membranes. The bladder was measured in three dimensions, and the product of these measurements was multiplied by $\pi/6$. The fetal bladder was observed with ultrasonography over a period of 30 to 60 minutes for each determination. The values for two determinations were averaged in determining urine output. In the study group the estimations were made an average of 3.4 ± 1.7 days (range 0.5 to 7 days) after hospitalization for ruptured membranes, while patients were in the hospital at bed rest. The control group consisted of low-risk pregnant patients, matched for gestational age, with singleton gestations and no medical complications, who were recruited from our obstetric practice. In the women with intact membranes estimations were made after rest for at least 30 minutes. No patients in the study were taking medications, other than prenatal vitamins and iron.

Statistical analysis between groups was done with Student's t test for variables with equal but unknown variances or by use of the method of least squares for simple linear regression, where appropriate.

Table I. Ultrasonographically determined fetal urine output

	Ruptured membranes	*Intact membranes*
Estimated weight (gm)	1616	1679
Urine output (ml/kg/hr, mean ± SEM)	11.5 ± 5.3	10.8 ± 4.3*

*$t = -0.36$; $p > 0.50$.

Results

Fetal urine output in the women with ruptured membranes (11.5 ± 5.3 ml/kg/hr) was not significantly different from the mean fetal urine output in the control group (10.8 ± 4.3 ml/kg/hr, Table I). Furthermore, there was no significant correlation between amniotic fluid index and fetal urine output ($r = -0.22$, $p = 0.49$). A power analysis showed that the sample size was sufficient to detect a 50% reduction in fetal urine output in the premature rupture of membranes group with a type II error of <20%.

Comment

The prognosis for the fetus in pregnancies complicated by preterm premature rupture of membranes may be related to residual amniotic fluid volume. Factors that influence residual amniotic fluid volume after rupture of membranes are poorly understood. Fetuses in pregnancies complicated by premature rupture of

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of North Carolina School of Medicine.
Received for publication June 8, 1990; accepted July 20, 1990.
Reprint requests: William J. Watson, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of North Carolina at Chapel Hill, CB 7570 MacNider, Chapel Hill, NC 27599-7570.
6/1/24037

membranes may have an increased risk of intrauterine growth retardation. Wladimiroff and Campbell[2] studied urine production in fetuses with intrauterine growth retardation and found that urine output was much lower than that expected for a normally grown fetus in three of 12 determinations.

In this study fetal urine output in pregnancies complicated by premature rupture of membranes was not reduced and did not vary as a function of residual amniotic fluid volume. Fetal urine production was unchanged even in the presence of severe oligohydramnios. We conclude that residual amniotic fluid volume after premature rupture of membranes does not depend on, or alter, fetal renal function. Residual amniotic fluid volume may depend on mechanical factors, such as maternal position, the site of leakage, and the size of the defect in the fetal membranes.

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The diagnosis of ovarian cancer by pathologists: How often do diagnoses by contributing pathologists agree with a panel of gynecologic pathologists?

Carl W. Tyler, Jr., MD,[a] Nancy C. Lee, MD,[b] Stanley J. Robboy, MD,[c] Robert J. Kurman, MD,[d] Allen L. Paris, MD,[e, f] Phyllis A. Wingo, MS,[b] and G. David Williamson, PhD[g]

Atlanta, Georgia, Newark, New Jersey, Baltimore, Maryland, and West Plains, Missouri

The Cancer and Steroid Hormone Study, a multicenter, population-based, case-control study of ovarian, breast, and endometrial cancer in women 20 to 54 years of age, permitted the diagnoses of contributing pathologists to be compared with those of a panel of three gynecologic pathologists. A diagnosis of ovarian cancer was made by contributing pathologists on 477 subjects. Agreement between the two groups of pathologists was 97% for primary epithelial ovarian cancer and 89% for primary nonepithelial ovarian malignancies. Agreement on diagnosis of major cellular subtypes of ovarian malignancy ranged between 73% for endometrioid cancer and 100% for clear cell carcinomas. We conclude that the diagnosis of pathologic features of primary ovarian cancer is highly predictable. Nonetheless, diagnosis by histologic type varies sufficiently that a review process should be considered for clinical or investigative decisions involving specific histologic diagnoses of ovarian cancer. (Am J Obstet Gynecol 1991;164: 65-70.)

Key words: Ovarian neoplasms, diagnosis, predictive value of tests, pathology

From the Office of the Director, Centers for Disease Control,[a] Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control,[b] Department of Pathology, New Jersey Medical School,[c] Departments of Gynecology, Obstetrics and Pathology, Johns Hopkins Hospital,[d] Center for Environmental Health and Injury Control, Centers for Disease Control,[e] Department of Pathology, Ozarks Medical Center,[f] and Epidemiology Program Office, Centers for Disease Control.[g]

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Reprint requests: Carl W. Tyler, Jr., MD, Centers for Disease Control, Mailstop E-42, Atlanta, GA 30333.
6/1/24575

Ovarian cancer is a significant cause of death for women in the United States. In 1989 an estimated 20,000 American women were newly diagnosed as having ovarian cancer, and 12,000 died from this malignancy.[1] The diagnosis of ovarian cancer by pathologists not only guides patient treatment but also provides the basis for evaluation of different approaches to treatment in multicenter clinical trials and in epidemiologic studies of risk factors. As an illustration, if a cancer that metastasized to the ovary were not diagnosed correctly on a pathology report, the patient might be treated as if the tumor were primary in the ovary when another site was the one of origin; this might lead to inappro-

priate therapy. If this occurred often enough, studies that evaluate surgical or chemotherapeutic approaches to primary ovarian cancer would incorrectly include tumors from other organs. This problem would also extend to epidemiologic investigations of the causes of ovarian cancer.

In the period 1980 to 1982 the Centers for Disease Control, with support from the National Institute of Child Health and Human Development and from the National Cancer Institute, conducted the Cancer and Steroid Hormone Study, a multicenter, population-based case-control study of ovarian, breast, and endometrial cancer in women 20 to 54 years of age.[2, 3] The study provided the opportunity to compare the diagnoses of ovarian malignancies made by contributing pathologists with those of a panel of three gynecologic pathologists. In this article we describe a measure used to make quantitative comparisons of the diagnoses, give a brief review of the methods used in the Cancer and Steroid Hormone Study, and report our findings.

Background

The accuracy of diagnostic tests, as exemplified by surgical pathology reports, has always been important. Even though we have always known that no diagnostic test is perfect, most of us assume that if an accurate diagnostic test for a particular condition does exist, it must be one reported by a skilled pathologist. Nonetheless, scientific articles have documented discrepancies among pathologists in the diagnosis of ovarian cancer.[4-7]

Investigators now quantify these discrepancies. In this report we will focus on the predictive value positive[8] as the measure for evaluation of diagnoses of histopathologic features of ovarian cancer. The predictive value positive is a measure of the probability that disease exists, given that the report of a diagnostic test is positive. It can be calculated with a numerator of patients known by a widely accepted, highly accurate standard to have ovarian cancer, who also have a histopathology report positive for ovarian cancer. The denominator is all the histopathology reports of ovarian cancer, regardless of whether all the patients included have disease. (Other measures of the characteristics of diagnostic tests include sensitivity, specificity, and predictive value negative. They are not included here because they require information about negative test reports and the absence of disease not reflected in the data we analyzed.)

In analyzing the predictive value positive of reports for a disease, the reports of interest (in this case, those of ovarian cancer) need to be compared with an acceptable standard. In this analysis we compared the reports of ovarian cancer made by all pathologists in the geographic area of each of the Surveillance, Epidemiology, and End Results (SEER) centers who contribute their reports (designated as contributing pathologists hereafter) with the consensus diagnosis of the panel of pathologists. In calculating the predictive value positive, the reports of the contributing pathologists serve as the denominator. Those confirmed by the panel are the numerator. Therefore, if 50 cases were reported to be ovarian cancer by SEER centers on the basis of the reports given them by contributing pathologists, but the panel confirmed only 45 of them, then the predictive value positive would be as follows:

$$PVP = 45/50 \times 100 = 90\%$$

Methods

The general methods of the Cancer and Steroid Hormone Study have been described previously.[2, 3] Subjects included in this analysis were enrolled through population-based tumor registries participating in the SEER program of the National Cancer Institute in six areas of the United States in which both interviews and retrieval of histopathology reports could be done (the metropolitan areas of Atlanta, Detroit, San Francisco, and Seattle, and the states of Connecticut and Iowa).

The subjects of this analysis were all women with primary ovarian cancer 20 to 54 years of age who resided in any of the reporting areas and who were first diagnosed as having ovarian cancer in the period Dec. 1, 1980, to Dec. 31, 1982. Of the 816 women who satisfied the case definition for ovarian cancer in the original Cancer and Steroid Hormone Study, 579 (71%) were interviewed. Slide retrieval was attempted only for the interviewed study subjects. Because only the six SEER centers mentioned in this article participated in retrieval of the slides of histologic specimens, the 40 women with ovarian cancer interviewed for the study from the other two centers (those in New Mexico and Utah) were excluded from this analysis. Furthermore, 62 additional subjects could not be included for the following reasons: slides could not be retrieved (18), inadequate specimens (8), possible previous history of ovarian cancer (4), and original diagnosis of the contributing pathologist not available (32). Therefore the subjects in this analysis included 477 women with primary ovarian malignancy as diagnosed by pathologists participating in the aforementioned population-based cancer registries.

All slides of histopathologic specimens were initially read by the contributing pathologists, and the results were then reported to the SEER centers. Because the tumor registries are population-based, the pathologists making these initial readings represent those practicing in the entire community.

Slides that were the basis for the original diagnosis made in the community in which the study subjects

Table I. Histopathologic diagnosis by contributing pathologists compared with those of a panel of gynecologic pathologists, by histologic category of ovarian tumor*

Contributing diagnoses	*Total*	*Benign*	*Panel diagnoses†*		
			Epithelial cancer	*Nonepithelial cancer*	*Metastatic cancer*
Epithelial cancer	432	1	420	2	9
Nonepithelial cancer	45	1	4	40	0
TOTAL	477	2	424	42	9

*A total of 62 subjects are excluded from this table because of the following reasons: 32 diagnoses not available, 18 slides not retrieved, 8 inadequate slides, and 4 subjects with a previous or uncertain history of ovarian cancer.

†Predictive value positive for all ovarian primary cancers [(424 + 42)/477] = 98%; predictive value positive for epithelial ovarian cancer (420/432) = 97%; predictive value positive for nonepithelial ovarian cancer (40/45) = 89%.

lived were then reviewed by a panel of three pathologists expert in the diagnosis of histopathologic features of ovarian cancer (Drs. Robboy, Kurman, and Paris). When required the panel requested additional slides for review. The panel reviewed each histologic specimen independently. If there were differences in the reviews of panel members, individual specimens were reviewed in greater detail with a microscope with eyepieces that permitted the panelists to examine and discuss each specimen simultaneously until consensus was reached. No written record of such differences were kept by the panel because the differences occurred so infrequently.

Each ovarian tumor was categorized by the panel as malignant (primary), malignant (metastatic), or benign. The malignant primary tumors were then classified as epithelial or nonepithelial, and assigned a histologic type including characterization as malignant or low malignant potential (borderline). Tumors of low malignant potential were grouped with malignant tumors when analyzed by histologic type. The classification scheme used was that of the World Health Organization.[9] In analyzing differences, trivial discrepancies in wording, such as papillary serous rather than serous, were not considered different. Further, as many ovarian tumors show blends of features (e.g., serous and endometrioid) the panel attempted not to consider such diagnoses as different from those reported by the contributing pathologists unless the discrepancy was readily apparent. In 79 cases a substantial portion of the tumor warranted a second diagnosis. Both diagnoses were considered in assessing concurrence between contributing and panel pathologists.

Statistical differences between predictive value positives were evaluated with χ^2 tests.[10] These differences were regarded as significant for $p < 0.05$.

Results

Among the women with primary ovarian tumors, approximately one third (35%) were in the age range 20 to 39 years; one third (34%) 40 to 49 years; and one third (32%) 50 to 54 years at the time of diagnosis. (Design of the Cancer and Steroid Hormone Study excluded women older than 54 years.) Almost 90% (88%) were white. Slightly more than four fifths (83%) were high school graduates. Approximately one quarter (26%) were nulliparous. More than 40% were no longer menstruating regularly.

In this investigation, the contributing pathologists diagnosed 477 tumors as primary ovarian cancer, and the panel pathologists agreed with 466 (98%), as shown in Table I. The predictive value positive for epithelial cancers (97%) is significantly greater than that for nonepithelial neoplasms (89%, $p < 0.05$). For cancer of low malignant potential (borderline), the predictive value positive is 93%, whereas that for invasive cancer is 85% ($p < 0.05$, Table II).

Contributing pathologists and the panel of gynecologic pathologists agreed on the histologic type of ovarian cancer less often than they did on the diagnosis of malignancy. In this analysis we considered four specific types of epithelial ovarian cancers. There were too few cases of the other types of epithelial ovarian cancer to be included. Table III shows the panel agreed with the 21 cases originally diagnosed by the contributing pathologists as clear cell carcinoma. The remaining three histologic types—serous, mucinous, and endometrioid—all had predictive value positives significantly lower than that for clear cell cancers ($p < 0.05$), but not different from each other. The two types of nonepithelial cancer—germ cell and sex cord—had predictive values of 92% and 88%, respectively; these values are not significantly different from each other or from the predictive value positives for the serous, mucinous, or endometrioid tumors.

The disagreements between the contributing pathologists and the expert panel focused on three issues. First, of the 11 instances in which the contributing and panel pathologists did not agree on the diagnosis of primary ovarian cancer (Table I), nine were diagnosed as metastatic tumors by the panel. Next, of the 63 cases in which disagreement occurred on the diagnosis of

Table II. Histopathologic diagnoses of ovarian cancers by contributing pathologists compared with those of a panel of gynecologic pathologists, by low malignant potential (borderline) and invasive cancer*

Contributing diagnoses	*Total*	*Predictive value positive (%)*	*Panel diagnoses*	
			Agree	*Do not agree*
Low malignant potential (borderline)	86	93	80	6
Invasive cancer	378	85	321	57

$p < 0.05$.

*Excludes 11 cases judged benign or metastatic and two diagnoses not available.

Table III. Histopathologic diagnoses by contributing pathologists compared with a panel of gynecologic pathologists by histologic type of ovarian cancer*

Contributing diagnoses	*Total*	*Predictive value positive (%)†*	*Panel diagnoses*	
			Agree	*Do not agree*
Serous	256	80	205	51
Mucinous	85	87	74	11
Endometrioid	52	73	38	14
Clear cell	21	100	21	0
Germ cell	24	92	22	2
Sex cord	17	88	15	2
Overall	455	82	375	80

*A small number of other types of epithelial (8) and nonepithelial (3) ovarian cancers are not included in this tabulation. The 11 metastatic and benign tumors shown in Table I are also excluded from this table.

†See text for discussion of the significance of the differences in predictive value positive values.

low malignant potential or borderline (Table II), the panel made a diagnosis of low malignant potential rather than invasive cancer in 57 (90%) and a diagnosis of invasive malignancy in six (10%) instead of low malignant potential. In this group of 63 cases, there was substantial concurrence on cell type. Finally, analysis of histologic type showed that the panel did not agree with the diagnosis of the contributing pathologists in 85 instances. In more than half of these cases (48 or 56%) the disagreement involved the distinction between endometrioid and serous cancer. In 36 instances the diagnosis of contributing pathologists was serous carcinoma, whereas the diagnosis of the panel pathologists was endometrioid cancer; the diagnoses were made in the reverse order for the other 12 instances.

An analysis of interview data showed that differences in diagnoses between the contributing pathologists and the panel of experts were not related to geographic region, age, race, menopausal status, estrogen use, use of oral contraceptives, or history of infertility.

Comment

This analysis highlights three findings. First, a diagnosis of primary ovarian cancer by contributing pathologists agreed with that of the panel in 98% of cases included in the Cancer and Steroid Hormone Study. In fact, of 477 cases reported as ovarian cancer, only two were judged by the panel to be benign; the remaining nine cases were diagnosed by the panel as metastatic (i.e., not primary ovarian cancer). Next, tumors of low malignant potential and invasive cancer were also differentiated with high (93% and 85%, respectively), albeit significantly different, levels of agreement. Of the cases on which there was disagreement, the panel diagnosed 90% (57 of 63) as tumors of low malignant potential rather than invasive cancer. Finally, the panel agreed with the contributing pathologists on 100% of cases reported to be clear cell ovarian carcinoma, but overall agreement on histologic type of tumor (375 of 455, or 82%) did not reach the same level attained in the correct diagnosis of primary ovarian cancer, or in the differentiation of tumors of low malignant potential from invasive cancer.

There are several limitations of this analysis. First, it is based on data from a study that focused on the epidemiologic relationship of cancers of the ovary, breast, and endometrium with administration of steroid hormone to women 20 to 54 years old. Therefore questions concerning ovarian cancer pathologic findings were not of highest priority. If they had been, the study design would almost surely have been different (e.g., it might have included women of all ages). Next, the pathologists on the panel knew the original diagnoses made by the contributing pathologists. We cannot be sure that this introduced bias into our results, but if it did it probably increased the predictive value positive. Fi-

Table IV. Summary of reports from the recent literature showing predictive value positive estimates

First author	*Year*	*Comment*	*Tumor characteristics*	*No.*	*Predictive value positive (%)*
Baak[4]	1982	Panel of 4 pathologists	Mucinous ovarian tumors	49	76
Cooke[5]	1984	Quality control survey	Mucinous ovarian cancer, low malignant potential	175	70
Hernandez[6]	1984	Panel of 2 pathologists	Ovarian epithelial cancer		
			Histologic type	68	60
			Histologic grade	68	60
Cramer[7]*	1987	Panel of 7 surgical pathologists	Ovarian epithelial and nonepithelial cancer		
			Mucinous	14	43
			Serous	25	32
			Clear cell	15	20
			Endometrioid	30	17

*Predictive value positive for this study is taken as the authors' "unanimity or near-unanimity (at most, one discordant pathologist)" category.

nally, the predictive value positive gives only part of the picture. Whereas all 21 reports of clear cell carcinoma made by the contributing pathologists had the concurrence of the expert panel, the panel members diagnosed 13 other clear cell malignancies that the contributing pathologists did not.

In comparing our results with those reported earlier, we found that each of the reports dealt with a different approach to investigation of the accuracy of cancer diagnosis (see Table IV). In addition, three of them focus more attention on mucinous carcinoma than the analysis reported here. The work of Baak et al.,[4] Hernandez and his colleagues,[6] and Cramer and his coworkers[7] each involved the comparison of individual members of a group of expert pathologists (four, two, and seven experts, respectively). It is important to emphasize that whereas the predictive value positives differ from study to study, differences in study design and clinical information known by the pathologists limit the value of comparing these investigations in detail. The specimens reviewed in the Cancer and Steroid Hormone Study were collected in a 2-year period that ended in December 1982; it is possible that the predictive value positive for the diagnosis of ovarian cancer by pathologists might have improved since that date. The studies cited here all have predictive value positive estimates that are near or less than those reported from the Cancer and Steroid Hormone Study. This suggests that if a change occurred, it was not appreciable; nonetheless, differences in study design limit the value of such a comparison.

The approach taken to validate the diagnosis of primary ovarian cancer in our analysis is more like that used in a study that assessed the diagnosis of lymphomas.[11] Nonetheless, the variability in the diagnoses by pathologists of these hematologic malignancies was substantial, ranging from 52% to 89%. The investigators concluded that variability in the diagnoses by pathologists decreases as an observer's experience increases and that the variability is least among expert pathologists.[11]

The statistical approach in some studies[7] of this problem make use of the κ test. Fleiss[10] points out that the κ statistic is suitable when all those making a set of observations are equally experienced; otherwise, he states, other tests may be more appropriate. Maclure and Willett,[12] in their assessment of 13 papers in which the κ test had been used, point out that it is ". . . a measure of agreement between two observers classifying subjects into two nominal categories. . .." They state that under circumstances like those in this analysis of ovarian cancer, alternative analytic approaches would be more appropriate.

These results from the Cancer and Steroid Hormone Study indicate that primary ovarian cancer is diagnosed with a very high level of predictability in the United States. Nonetheless, cancer that metastasizes to the ovary made up a large proportion of the very small percentage of malignancies that are not correctly diagnosed. For those few patients in whom this occurs, the consequences may be serious because the primary cancer could go untreated. In addition, this analysis indicates that ovarian cancer of low malignant potential is interpreted as invasive malignancy in almost one of every eight cases (57 of 464, or 12%) of primary ovarian cancers, as shown in Table II. To the extent that this leads to treatment that has more frequent or more serious side effects, this problem with diagnosis also influences patients with this disease.

These results lead us to recommend that research on the different histologic types of ovarian cancer be reviewed by a panel of pathologists experienced with the

diagnosis of gynecologic disorders. The analysis of clear cell ovarian cancer supports our concern. Whereas the panel agreed wth the contributing pathologists on all 21 clear cell ovarian cancers, the panel found 13 other instances in which tumors reported as endometrioid were reclassified by the panel as clear cell ovarian cancer, indicating that the histologic type of 38% (13 of 34 clear cell cancers) of malignancies with this cell type was revised as a result of the review.

Guidance for clinical practitioners is more difficult to suggest. Although ovarian cancer that has metastasized to the ovary from some other primary site is rare, the possibility of a distant primary site is the first and most serious factor to be considered in the diagnosis of ovarian cancer. In addition, if treatment will be influenced by the histologic type of primary ovarian cancer, review of such specimens by competent colleagues (even if only one is available) deserves serious consideration.

No matter what role a physician plays in caring for women at risk of ovarian cancer, their survival depends on correct identification of the origin of the malignancy and may be influenced by histologic type as well as by clinical characteristics. For these reasons, the precise diagnosis of ovarian cancer by pathologists is important for researchers, clinicians, and women with this condition.[13]

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Spontaneous ovarian-to-vaginal fistula: A case report

Plaul Claman, MD,[a] Mark Dover, MD,[b] Raphael Saginur, MD,[c] Hardy Tao, MD,[d] and Manuel Orizaga, MD[e]

Ottawa, Ontario, Canada

A 59-year-old nulligravid woman presenting with resistant vaginitis was found to have a spontaneous ovarian-to-vaginal fistula arising from a benign ovarian abscess. (Am J Obstet Gynecol 1991;164:71-2.)

Key words: Fistula, ovarian to vaginal

Although ovarian-rectal fistula and intestinal-vaginal fistula have been reported, the occurrence of ovarian-vaginal fistula appears to be a rare event indeed. Previous cases reported involving ovarian-vaginal fistula were after vaginal hysterectomy[1] or in association with a cystic teratoma.[2]

Case report

A nulligravid, 59-year-old woman with type II diabetes presented with a 1-year history of a foul-smelling vaginal discharge that was unresponsive to multiple treatments.

Approximately 1 month before the onset of vaginal discharge, this patient was admitted to another hospital with abdominal pain, leukocytosis, and an ultrasonographic diagnosis of a right-sided pelvic abscess. After 2 weeks of parenteral antibiotics (ampicillin and tobramycin) the pain lessened and the abscess had halved in size on ultrasonographic examination. The patient was discharged home on a regimen of oral antibiotics (cephalexin) with plans to be readmitted 3 months later for interval appendectomy. After hospital discharge (about 2 months before the appendectomy) she began to notice this foul-smelling vaginal discharge. The discharge was copious enough to require the use of up to two dozen vaginal pads daily. An appendectomy neither changed the nature of discharge nor cured the still persistent problem of abdominal pain. Pathologic examination of the appendix showed some periappendiceal inflammation and a small carcinoid tumor of the mucosa and submucosa, which was not considered significant.

Over the ensuing 9 months her family doctor prescribed multiple courses of tetracycline, metronidazole, antifungal cream, and AVC (sulfanilamide, aminacrine, allantoin) cream. Although the vaginal discharge would transiently improve with tetracycline, it returned within days of stopping this medication. Medical history was otherwise unremarkable.

When she was referred to us, we found her to be a moderately obese normotensive woman in no physical distress. Findings of general physical examination were unremarkable except that there was some tenderness to deep palpation in the right lower abdomen. On pelvic examination the vulva was normal although somewhat erythematous. The vagina was also normal with healthy rugations although there was a purulent yellowish foul discharge. Microscopic examination with potassium hydroxide and saline solution preparations were negative for clue cells, yeast, and trichomonas. On careful examination of the vaginal vault there appeared to be a possible fistulous opening at the cervical-vaginal junction at the 7 o'clock position (just medial to the uterosacral ligament insertion area). Because of good uterine support and obesity this fistulous tract could not be clearly seen or cannulated. A computerized tomographic scan of the pelvis demonstrated a thick-walled cystic structure 5 cm in diameter in the right lateral aspect of the pelvis at the level of the inferior portion of the right sacroiliac joint. A percutaneous injection of contrast media through a 22-gauge needle was done under fluoroscopic control, which demonstrated clear evidence of a cavitating space with a small lumen fistula approximately 2.5 cm long that was communicating with the right lateral aspect of the vaginal fornix (Fig. 1). Methylene blue dye was then injected into the abscess cavity and was subsequently found in the vagina on physical examination.

With these data a laparotomy was performed. Findings at operation included minimal adhesions with a normal uterus. Both ovaries and tubes, however, were involved in dense adhesions, with the adnexa bilaterally bound to the lateral pelvic sidewalls. The left ovary in itself was normal. The right ovary, however, was cystically enlarged to approximately 4 cm in diameter and stuck to the vaginal fornix with an apparent fistulous tract. After the ovary was dissected free, there was a definite opening into the vaginal fornix. This was curetted clean and the fistulous opening was closed with a purse-string suture of 2-0 proline. An oophorectomy

From the Departments of Obstetrics and Gynecology,[a] Surgery,[b] Medicine,[c] Radiology,[d] and Pathology,[e] Ottawa Civic Hospital, University of Ottawa.

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Reprint requests: Paul Claman, MD, Ottawa Civic Hospital, Department of Obstetrics and Gynecology, 1053 Carling Ave., Ottawa, Ontario, Canada K1Y 4E9.

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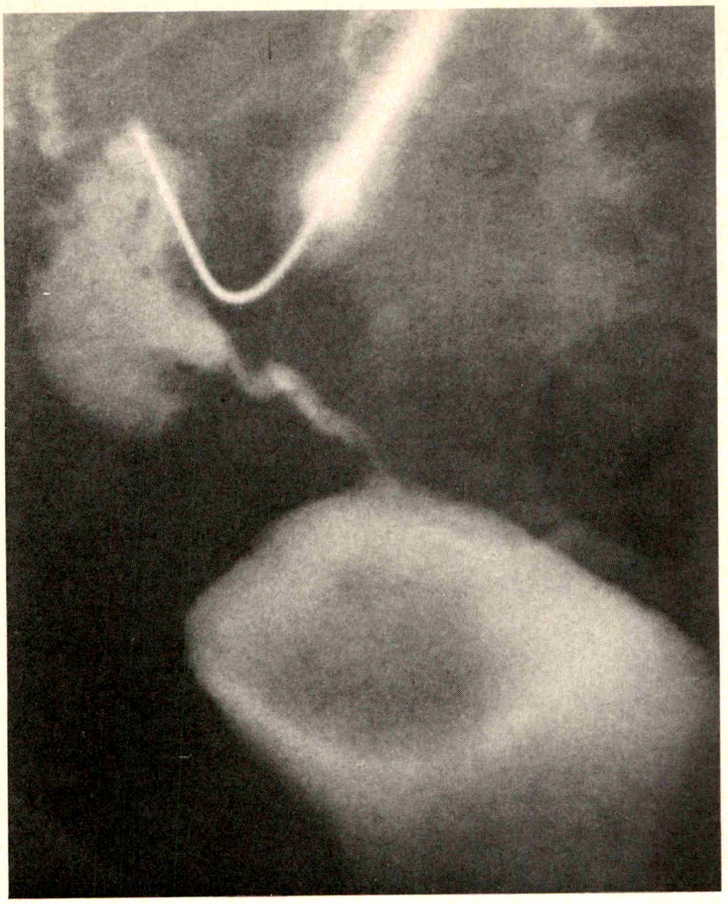


Fig. 1. Fluoroscopically controlled percutaneous injection of contrast media into the abscess cavity. A 3 cm fistula is noted between the cavity and the vaginal fornix. Before removal of the 22-gauge needle, methylene blue dye was injected into the cavity, which was then found in the vaginal vault on physical examination.

was performed. The final histologic diagnosis was consistent with a benign ovarian abscess. After operation the patient noticed immediate resolution of the vaginal discharge. Her recovery was complicated only by a subcutaneous wound infection, which was treated with drainage and antibiotics. Culture of the ovarian abscess did not show significant bacteria, probably because the patient had received preoperative antibiotics (gentamicin, metronidazole, and penicillin).

Comment

This is the first report of a spontaneous ovarian vaginal fistula associated with a benign ovarian abscess that was likely because of chronic pelvic inflammatory disease (as opposed to a complication of surgery or a cystic teratoma). Perhaps this patient's diabetes put her at higher risk for chronic infection with this distinctly unusual entity of ovarian-vaginal fistula, where the ovary, which was adherent to the vaginal fornix, had drained into the vaginal fornix via a fistulous tract.

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Relationship of laparoscopic findings to self-report of pelvic pain

Anna L. Stout, PhD,[a] John F. Steege, MD,[b] William C. Dodson, MD,[c] and Claude L. Hughes, MD, PhD[c]

Durham, North Carolina

An assessment battery including standardized measures of behavioral and psychosocial factors associated with other chronic pain conditions was administered to 102 women scheduled for laparoscopic surgery. Surgeons who were blinded to the patient's self-reported pain data completed the American Fertility Society classification for endometriosis and adhesions on the basis of observed physical disease. Although American Fertility Society classification scores were significantly related to self-assignment into pain or no-pain groups, the extent of physical disease evaluated by this procedure was not significantly correlated with ratings of pain levels or a number of indexes of impairment. The group of patients with laparoscopically diagnosed pathologic conditions reported higher pain levels and greater interference than the group who reported pain and had negative laparoscopic results; however, some women with observable pathologic conditions reported no pain symptoms. (AM J OBSTET GYNECOL 1991;164:73-9.)

Key words: Chronic pelvic pain, pain assessment, laparoscopic surgery

The complaint of chronic pelvic pain is one of the most frustrating and controversial areas of gynecologic practice. Although a number of possible organic conditions have been considered as etiologic factors, no physical abnormality can be documented in many women who report chronic pelvic pain. Early reports in the medical literature implied that women who reported chronic pelvic pain had a high degree of emotional disturbance such as feminine identity problems arising from conflicts regarding adult sexuality[1]; psychiatric disturbance characterized by mixed character disorders with predominant schizoid features[2]; and high neuroticism and unsatisfactory relationships.[3] Duncan and Taylor[4] and Benson et al.[5] reported an association between the onset of symptoms of pelvic pain and emotional stress.

These earlier published reports of the presence of pelvic pain symptoms in the absence of clear organic disease have been questioned from several perspectives. Because physical abnormalities that cannot be identified on pelvic examination can often be observed during laparoscopic surgery, the women who were considered to have normal pelvic examinations may have had some type of organic pathologic condition if evaluated by current procedures. Cundnan et al.[6] reported findings on nearly 1200 patients during diagnostic laparoscopy for pelvic pain. In their series, 63% of the women with normal pelvic examinations before diagnostic laparoscopy had abnormal findings on diagnostic laparoscopy. On the other hand, questions have been raised with regard to the assumption of a cause-and-effect relationship between physical findings and pain symptoms. Kresch et al.[7] identified some possible pathologic condition in 29% of asymptomatic women who underwent laparoscopic surgery for tubal ligation.

Other methodologic criticisms that raised questions with regard to the conclusions from previous studies suggesting the high prevalence of psychopathology in chronic pelvic pain patients are as follows: (1) selection bias in the sample of patients who were studied psychologically because of high refusal rates for psychological assessment that are reported in some studies; (2) potential bias of psychological evaluators who were not blinded to the presence or absence of identified organic disease; and (3) lack of appropriate control groups to establish population base rate data for problems such as sexual adjustment difficulties. In all studies of which we are aware, limited information is available on the psychological status of these women before pain onset. Several more recent investigations[8-10] reported that women who had pelvic pain with positive laparoscopy findings and negative laparoscopic findings

From the Division of Medical Psychology, Department of Psychiatry, and Department of Obstetrics and Gynecology,[a] the Division of Gynecology, Department of Obstetrics and Gynecology,[b] and the Division of Endocrinology and Infertility, Department of Obstetrics and Gynecology,[c] Duke University Medical Center.

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Reprint requests: Anna L. Stout, PhD, Box 3263, Duke University Medical Center, Durham, NC 27710.

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exhibited similar psychological profiles, raising the question of whether pain experience itself produces the emotional and sexual difficulties associated with chronic pelvic pain.

Current conceptualizations of chronic pain emphasize the complexity of the chronic pain condition. The pain experience appears to be a product of more than tissue disease. Behavioral and psychological factors are increasingly recognized as important contributors to the understanding and treatment of chronic pain syndromes. The conditions of chronic back pain and chronic headache pain have been the most extensively studied. Variables such as limitation of activities, ratings of pain severity, pain descriptions, administration of medication, and perception of control have been shown in numerous studies to predict functional disability and response to medical treatment often to a greater extent than organic pathologic factors identified in these conditions.

The two most common physical abnormalities that are suspected in the organic cause of chronic pelvic pain are endometriosis and adhesions. The extent to which endometriosis and adhesions can account for chronic pelvic pain remains controversial. As previously cited, Kresch et al.[7] discovered endometriosis or adhesions in 29% of asymptomatic women during laparoscopic surgery for tubal ligation. Rapkin[11] found laparoscopically diagnosed evidence of pelvic adhesions in 26% of patients with chronic pain and 39% of infertility patients; however, only a very small portion of the infertility patients with pelvic adhesions complained of pelvic pain. In clinical practice, many gynecologists have reportedly observed patients with a few small endometriosis implants or adhesions who report significant pain, whereas other patients who are found to have significant disease may not describe pain symptoms.

In their review of behavior assessment and treatment of chronic pain, Keefe and Gil[12] offer several suggestions for future assessment research including recommendations for the use of standardized assessment of behavioral and psychosocial factors and standardized methods for quantification of the severity of the tissue disease basis for pain. The purpose of this preliminary investigation is to explore the relationship between findings of endometriosis and adhesions documented by laparoscopic surgery with a standardized structured classification system and the self-report of pain parameters on selected standardized psychological instruments. Information from this study will be used to guide the direction of future research. More specifically, if organic status is found to be highly correlated with pain behavior and functional status, medical-surgical approaches to organic pathologic conditions would appear to be the most effective treatment. However, if impairment cannot be accounted for on the basis of organic pathologic findings identified by the currently accepted medical diagnostic procedures, further systematic evaluation of psychosocial factors seems clearly mandated.

Material and method

Subjects. The subjects were 102 women scheduled for laparoscopic surgery for diagnostic or treatment purposes by gynecologists on the faculty of Duke University Medical Center. Subjects were recruited from the Division of Reproductive Endocrinology and Division of Gynecology of the Department of Obstetrics and Gynecology. A screening questionnaire inquired with regard to demographic variables, marital satisfaction, gynecologic concerns, reason for laparoscopy, and the presence or absence of dysmenorrhea, pelvic pain other than during menses, and dyspareunia. Indications for having laparoscopic surgery were as follows: to investigate causes of pain, 64%; to investigate causes of infertility, 35%; to investigate abnormal findings from other examinations or tests (pelvic examinations, sonograms), 19%; and for sterilization only, 15%. With the exception of the sterilization group, some subjects were undergoing laparoscopic surgery for more than one reason.

Procedures. Approval for the study was obtained from the Institutional Review Board before initiation of the protocol. Subjects scheduled for laparoscopy were identified from the operating room schedule and arrangements were made to contact the subjects about participation in the study at the time of preoperative evaluation. Informed consent was obtained at that time. Subjects completed and returned the questionnaires before the laparoscopic surgery and before operative physical findings were discussed to eliminate the influence of physical findings on their responses.

The surgeons of the patients who agreed to participate in the study were asked to complete the American Fertility Society classification for endometriosis and adhesions[13] with clarification to score for the presence of adhesive disease regardless of presumed cause. The only exception to the scoring rules was that scores for pathologic conditions in a remaining adnexal area were not doubled if the other ovary or tube were absent because this weighting procedure seemed specifically related to fertility rather than to currently observed pathologic conditions. Surgeons were also asked to record and diagram on the American Fertility Society scoring form any other evidence of abnormality. These forms were then also scored by one of the authors (J.F.S.) for the presence or absence of disease to exclude the existence of pathologic conditions other than endometriosis or adhesions. Surgeons were blinded to the patient's self-reported questionnaire data related to pain symptoms.

Measures. Because this is an exploratory study of the relationship between self-report of chronic pelvic pain and physical findings, a battery of assessment measures was assembled that included some standardized evaluation instruments that have been used with other chronic pain populations and some original questionnaires were developed to assess specific characteristics of pelvic pain. The assessment battery included the following:

Pain Questionnaire for Women. The Pain Questionnaire for Women (Stout, unpublished data) is a pilot questionnaire designed to evaluate characteristics of pelvic pain including a diagram of the female torso for shading in pain location, questions related to reported symptoms of endometriosis and adhesions, association with the menstrual cycle and sexual activity, and questions regarding pain parameters, medication taken, and physical activity.

McGill Pain Questionnaire—Pain Rating Index. The McGill Pain Questionnaire[14] is a well-established and commonly used instrument with adequate psychometric properties. The rationale for the development of the McGill Pain Questionnaire has its basis in the gate control theory of pain.[15, 16] The gate control theory postulates that pain is a multidimensional concept with sensory, motivational and evaluative components. The Pain Rating Index of the McGill Pain Questionnaire is frequently extracted for use in the evaluation of pain and to track response to pain control interventions. The Pain Rating Index currently includes 20 adjective subclasses of two to six pain descriptions ranked according to intensity of pain. The values of the words chosen by a patient are then added to obtain a score for each of the following categories: sensory, affective, evaluative, miscellaneous, and a total score for all categories. In accordance with Pain Rating Index instructions, patients are not required to select a word from every subclass and are limited to choosing the one-word "best descriptor" in each subclass. The total number of words chosen was also calculated from the Pain Rating Index.

West Haven–Yale Multidimensional Pain Inventory, part I. The West Haven–Yale Multidimensional Pain Inventory[17] is a brief, psychometrically sound inventory designed to provide an evaluation of several aspects of a person's unique subjective experience of pain. Part I consists of six scales that were developed a priori to assess the following areas: (1) pain severity and suffering; (2) pain-related life interference including interference with family and marital functioning, work and work-related activities, and social-recreational activities; (3) dissatisfaction with present level of functioning in each of the subareas listed in area 2; (4) appraisal of support received from spouse, family, and significant others; (5) perceived life control, incorporating the perceived ability to solve problems and feelings of personal mastery; and (6) affective distress. Responses to each item are recorded on a seven-point scale ranging from 0 (no change) to 6 (extreme change).

Statistical analysis. Group comparisons for continuous variables were done with the Student t test or Wilcoxon two-sample test where appropriate. Spearman rank correlations were used to assess the relationship between self-reported pain variables, physical findings, and age. Because the Spearman rank correlation test does not adjust for weighted scores and it is unknown whether the weighting procedures for pathologic conditions in the American Fertility Society scoring system are relevant for pain symptoms, American Fertility Society total scores were collapsed to a 0 to 6 scale to match the range of other variables. This modified American Fertility Society total score was created by setting up a gradient of six levels of physical pathologic conditions on the basis of distribution of American Fertility Society total scores. Group differences for categoric variables were done with the χ^2 test, adjusting for inadequate cell sizes when appropriate.

Results

Pain versus no pain group. Of the 102 women who participated in the study, 88% reported on the screening questionnaire that they had some type of pelvic pain (dysmenorrhea, 90%; pain at other times during the menstrual cycle, 82%; and dyspareunia, 77%). The group of women who were self-assigned to the pain group ($N = 90$) were compared with the group who did not experience pelvic pain, no-pain group ($N = 12$) (Table I). There were no significant differences between the two groups with regard to age (both 32 ± 6 years), race, education, full-time employment, marital status, or global rating of marital satisfaction. In addition, no significant differences were found between the pain and no-pain groups in the number of previous surgeries, history of abdominal or pelvic surgery, motivation for laparoscopic surgery to investigate infertility, or the current or future desire for pregnancy. As might be expected, patients in the pain group indicated more frequently that investigation of pain, abnormal examination findings, or concern about endometriosis, adhesions, or sexually transmitted diseases were the motivations for the laparoscopic surgery.

The pain and no-pain groups were significantly different with regard to total American Fertility Society scores ($p \leq 0.004$). The pain and no pain groups were also significantly different with regard to the modified American Fertility Society total scores ($p \leq 0.01$), indicating the total scores on the American Fertility Society classification of endometriosis and adhesions were significantly related to patient's self-assignment into the pain or no-pain group. There was also a trend for endometriosis ($p \leq 0.09$) and adhesions ($p \leq 0.19$) to be

Table I. Comparison of self-reported pain group with no-pain group

	Pain (N = 90)	*No pain (N = 12)*
Age (yr)	32.2 ± 6.2	32.1 ± 6.0
No. of previous surgeries	1.2 ± 1.3	0.8 ± 1.2
American Fertility Society total score	7.2 ± 12.5*	1.6 ± 4.6*
Modified American Fertility Society score	2.2 ± 2.1*	0.7 ± 1.4*
White (%)	88	67
College grad (%)	36	42
Employed full-time (%)	78	83
Married (%)	74	67
Satisfied with marriage (%)	61	63
Having children (%)	47	75
Currently attempting pregnancy (%)	33	42
Desire future pregnancy (%)	59	56
Concern about sexually transmitted disease (%)	34*	8*
Concern about endometriosis (%)	62*	25*
Concern about adhesions (%)	52*	17*
Past abdominal or pelvic surgery (%)	56	42
Investigating infertility (%)	34	50
Investigating pain (%)	72*	0*
Investigating examinations/tests (%)	21*	0*

*$p < 0.05$.

documented more frequently in the pain group, but differences were not statistically significant.

Further analysis of information from the battery of questionnaires is limited to the pain group because women who did not report pelvic pain did not complete these instruments. Within the pain group, the relationship was explored between the modified American Fertility Society total score and a number of pain variables considered important in assessment of the impact of pain symptoms on the ability to function. The extent of physical disease classified by the modified American Fertility Society score was not significantly correlated with duration of the pain symptom, rated levels of dysmenorrhea, luteal or follicular phase pain, dyspareunia, pain frequency, duration of pain episodes, days in bed, or medication taken. McGill Pain Rating Index sensory, affective, evaluative, and miscellaneous subscale scores and McGill Pain Rating Index total scores were not significantly correlated with American Fertility Society scores of physical findings. The relationships between the extent of physical disease and areas assessed by part I of the West Haven–Yale Multidimensional Pain Inventory were also examined. The amount of physical disease observed at laparoscopy was not significantly correlated with Multidimensional Pain Inventory subscale scores of pain severity, interference, life control, affective distress, or support.

Table II. Diagnostic laparoscopy findings and self-reported pelvic pain symptoms

Physical pathologic condition	*Self-reported pain*		
	Present	*Absent*	*Total*
Present	71	7	78
Absent	19	5	24
TOTAL	90	12	102

The association of self-reported pain descriptions with physical findings observed during laparoscopy was also examined. The report of rectal, bladder, or radiating leg pain was not related to the findings of endometriosis in this sample, although the report of menstrual cycle variation of pain symptoms was marginally related to a finding of endometriosis ($p < 0.12$). The report of rectal or bladder pain was also not related to the finding of adhesions; however, radiating leg pain ($p < 0.15$) and menstrual cycle variations ($p < 0.13$) in pain showed a tendency to contraindicate adhesions. No association was found between the documentation of endometriosis or adhesions with pain worsened by bending, lifting, physical exercise, reaching, sitting, standing, lying down, walking, or bladder or bowel elimination.

Pathologic finding versus no pathologic finding. Findings of laparoscopic surgery were categorized into those with physical pathologic conditions and those with no physical pathologic conditions by a gynecologist blinded to the self-reported pain ratings and classified for location of disease into seven categories of the pelvic region as follows: right, left, central, bilateral, diffuse, right and central, and left and central. The boundary between unilateral and central areas was arbitrarily drawn between the umbilicus and approximately the medial aspect of the femoral canal. Of the 102 patients in this sample, 78 (76%) were considered to have some type of pathologic condition and 24 (24%) were considered to have negative laparoscopic findings. Nineteen of the 24 patients with negative laparoscopic findings reported pelvic pain symptoms (Table II). Further statistical comparisons were then made between the group with self-reported pain symptoms and negative laparoscopic findings ($N = 19$) and the group with self-

Table III. Comparison of group with pathologic condition present and group with absence of pathologic condition in patients with pain

	Range of possible scores	*Pathologic condition*	
		Present (N = 71)	*Absent (N = 19)*
Dysmenorrhea	0-6	4.2 ± 1.6*	3.4 ± 1.6*
Luteal phase pain	0-6	3.5 ± 1.7	2.9 ± 1.5
Follicular phase pain	0-6	3.0 ± 2.0*	1.7 ± 1.9*
Dyspareunia	0-6	2.7 ± 2.0	1.7 ± 2.0
Time in bed	0-6	2.0 ± 2.1*	0.8 ± 1.2*
Pain frequency	1-5	2.1 ± 1.5*	3.0 ± 1.6*
Months since onset	1-240	36.7 ± 52.0	27.6 ± 23.5
Duration of episodes	1-6	3.6 ± 2.0	3.1 ± 1.7
Medication frequency	0-5	2.0 ± 1.9	1.3 ± 1.4
McGill sensory subscale	0-42	15.1 ± 8.0	12.7 ± 8.3
McGill affective subscale	0-14	2.5 ± 3.0	1.6 ± 3.2
McGill evaluative subscale	0-5	2.6 ± 1.8*	1.7 ± 1.6*
McGill miscellaneous subscale	0-17	4.6 ± 4.2	3.8 ± 4.6
McGill total subscale	0-78	24.4 ± 13.9	20.1 ± 16.1
McGill number words	0-20	9.6 ± 5.1	7.8 ± 5.6
MPI severity	0-6	2.7 ± 1.6*	1.5 ± 1.3*
MPI interference	0-6	2.6 ± 1.9	1.1 ± 1.2
MPI life control	0-6	3.4 ± 1.2	4.0 ± 1.1
MPI affective distress	0-6	3.1 ± 1.5	2.7 ± 1.5
MPI support	0-6	3.9 ± 1.2	3.6 ± 1.5

MPI, Multidimensional Pain Inventory.

reported pain symptoms and positive laparoscopic findings ($N = 71$) (Table III). The group with laparoscopically diagnosed pathologic conditions had significantly higher rated levels of dysmenorrhea ($p \leq 0.04$) and follicular phase pain ($p \leq 0.02$), more time in bed ($p \leq 0.003$), higher scores on the McGill evaluative subscale ($p \leq 0.04$), and Multidimensional Pain Inventory pain severity ($p \leq 0.003$) and interference subscales ($p \leq 0.0001$). No other differences emerged between the two groups in their global assessment of the quality of marital relationship or any other variables assessed in this study.

The group ($N = 7$) with pathologic conditions identified at laparoscopy but who reported no pain symptoms was too small for statistical analysis but was of particular interest. American Fertility Society total scores in that group ranged from 0 to 16. Described pathologic findings included 10-week size fibroids ($N = 1$), minimal endometriosis ($N = 3$), and filmy and dense adhesions ($N = 3$).

The location of pathologic conditions observed during laparoscopic surgery was also compared with the location of pain indicated on the body maps of the female torso. With the seven categories of the pelvic region described in this article, there was an exact match between location of pathologic condition from laparoscopic surgery and self-reported location of pain in 25%; however, the self-reported pain location was completely outside the area of pathologic findings in only 3% of the cases. The remainder of the cases (72%) shaded in an area on the body map larger than the area of pathologic findings or had an area of disease larger than the indicated pain area. Five of the 19 women who reported pain but who did not have an identified pathologic condition described very diffuse pain.

Comment

Patients were included in this study if they indicated that they considered themselves to have pain during menstrual bleeding, in their pelvic or abdominal areas at other times during the menstrual cycle, or during sexual intercourse. Data were examined to determine whether women might qualify for the study on the basis of mild-moderate dysmenorrhea that other women might consider normal; however, no patients were included in the pain group who only reported dysmenorrhea at a level of ≤3 on a 0 to 6 scale. Nevertheless,

the broad inclusion criteria may have weakened relationships with more severe reports of pain. The present study is limited by the small number of patients without any pain symptoms; however, laparoscopic surgery is unlikely to be performed unless some suspicion of abnormality exists or for the purpose of sterilization. Whereas the present study does not represent a cross-section of the adult female population, it does use a prospective design feature of quantification of self-reported pelvic pain before laparoscopic inspection for diagnostic evaluation. Furthermore, because the operating surgeons were unaware of the pain questionnaire responses, observer bias should be minimized by the design.

Although physical pathologic conditions may produce sensory changes that many women label as pain, this study strongly suggests that the degree of pelvic organic disease as evaluated by the currently accepted diagnostic procedure (diagnostic laparoscopy) is at most minimally related to rated levels of pain symptoms and functional impairment. On the basis of these findings, the majority of women reporting pelvic pain symptoms may be expected to have some type of physical abnormality, primarily endometriosis or adhesions, observed during laparoscopic examination. The location of the abnormality almost always corresponds to the location of the pain. However, the contribution of these physical conditions to the pain may be relatively small in comparison with multiple other less easily assessed psychosocial factors. Within a group of women who reported pelvic pain, a significant portion (21% in this sample) showed no evidence of physical disease at laparoscopy. A few women in this sample who underwent laparoscopic surgery for reasons other than investigation of pain had comparable physical findings that were not associated with pain symptoms.

The minimal relationship between physical findings and severity of self-reported pain symptoms appears to suggest two possible explanations. The first might be that the American Fertility Society classification for endometriosis and adhesions is not applicable to the type of pathologic condition associated with pelvic pain; however, the feasibility of this explanation is certainly reduced by the fact that 19 of the 24 patients with negative laparoscopic findings on the American Fertility Society system were also not considered to have any abnormal pelvic findings (including other possible pathologic conditions) when reviewed by a gynecologist who was blinded to the patients' pain ratings. It is possible that these patients may have physical discomfort on the basis of some type of functional rather than anatomic process (i.e., prostaglandins), which cannot be identified by laparoscopic examination. Nevertheless, this group with normal laparoscopic findings who reported pain symptoms may be reduced as more sensitive medical or surgical diagnostic techniques are developed. Further exploration of methods for quantification of the severity of pelvic disease is planned.

Second, the evidence from this data strongly suggests marked individual differences in how persons experience or interpret physical sensory input from pelvic anatomy. Although the subsamples were too small for statistical analysis in this study, the two discordant subgroups (pain present—pathologic condition absent, pain absent—pathologic condition present) would be an interesting focus for future studies.

In other chronic pain conditions, psychological factors have been shown to account for a significant portion of these individual differences in functional impairment. Keefe et al.[18] found that depression was a significant predictor of pain behavior in patients with low back pain independent of demographic or medical status variables; however, psychological variables (depression, anxiety, and helplessness) were not found to be independent predictors of pain behaviors in rheumatoid arthritis patients[19] pointing out the danger of generalizing from one chronic pain condition to another. Depression evaluated by the affective distress subscale of the Multidimensional Pain Inventory was not a significant predictor of functional impairment in this sample; however, a possible relationship may have been obscured because the majority of women without pain symptoms were undergoing laparoscopic surgery for investigation of infertility and may also have been experiencing comparable depressive symptoms. Because patients without pain symptoms did not complete the Multidimensional Pain Inventory, this hypothesis cannot be evaluated from this data. Stout et al.[20] presented preliminary evidence that patients with pelvic pain may differ from other groups of women with chronic pain conditions on several psychosocial and behavioral variables. While awaiting the results of further research to delineate the impact of psychosocial factors on pelvic pain symptoms, clinical results may be improved by simultaneously restoring normal anatomy to the degree possible and administering or recommending therapeutic attention for emotional and psychosocial concerns.

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Phosphorus 31 magnetic resonance spectroscopy of human placenta and quantitation with perchloric acid extracts

Helen H. Kay, MD,[a, c] John D. Gordon, MD, Anthony A. Ribeiro, PhD,[c] and Leonard D. Spicer, PhD[b, c]

Durham, North Carolina

Phosphorus 31 magnetic resonance spectroscopic studies of fresh placental tissue are reported that indicate resonances for adenosine triphosphate, inorganic phosphate, sugar phosphates—phosphomonoesters, and phosphodiesters. Perchloric acid extract methods were used to further characterize and quantitate phosphorous metabolites in term human placentas by phosphorus 31 magnetic resonance spectroscopy. The perchloric acid extracts give enhanced resolution of phosphorus signals and allow identification of other phosphorus metabolites including small amounts of phosphocreatine. Emphasis was placed on quantitating adenosine triphosphate levels in the acid extracts with the use of the external reference standard hexachlorocyclotriphosphazene in a coaxial capillary system. Adenosine triphosphate levels measured in this way ranged from 0.404 to 0.709 μmol per gram wet weight. Comparison with an internal standard method with phosphocreatine is also reported. Contribution to the measured high-energy phosphate pool from blood in the highly vascularized tissue was found to be relatively large and could range from 30% to 50% of the total adenosine triphosphate measured. (AM J OBSTET GYNECOL 1991;164:80-7.)

Key words: Phosphorus 31 nuclear magnetic resonance spectroscopy, human placenta, adenosine triphosphate

Nuclear magnetic resonance spectroscopy is a technique that chemists have used to study chemical structure. More recently it has been used to characterize the behavior of molecules in biologic tissue.[1] Magnetic resonance spectroscopy uses the resonant frequencies of certain naturally occurring, nonradioactive nuclei (hydrogen 1, phosphorus 31, carbon 13) that resonate under the influence of an external magnetic field. The exact resonance frequency of the nuclear spin of an atom (e.g., ^{31}P) will depend on the magnetic environment set up by local electrons. This property, termed the chemical shift, allows the nucleus being studied to be identified within a molecule in a chemical solution or in a biologic tissue. The collection of resonance signals at their separate chemical shifts constitutes the magnetic resonance spectrum. The units of chemical frequency shift in the spectra are parts per million (ppm) or hertz.

From the Departments of Obstetrics and Gynecology,[a] Biochemistry,[b] and Radiology,[c] Duke University Medical Center.
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Reprint requests: Helen H. Kay, MD, Box 3373, Duke University Medical Center, Durham, NC 27710.
6/1/24392

The ability of magnetic resonance spectroscopy to monitor the atomic signals of molecules makes it an important tool for metabolic studies in a variety of organ systems in both human beings and animals.[2] Because it is considered a noninvasive procedure magnetic resonance spectroscopy may ultimately also become a very useful clinical tool. Magnetic resonance spectroscopy has recently been used in preliminary studies of the human placenta[3, 4] with promising results, suggesting that a better understanding of placental metabolism can be obtained with this technique.

Placental metabolism and function have been generally understudied even though the placenta plays an important role in the maintenance of fetal well being. Its primary functions are to transport nutrients (e.g., oxygen, amino acids, sugars, and lipids) to the fetus and to remove waste from the fetus. Another major function is synthesis of large amounts of steroid hormones, primarily estrogen and progesterone. Estrogens stimulate placental uptake and use of glucose as well as formation of glycogen and glycerol.[5] The placenta also actively synthesizes large amounts of proteins such as human chorionic gonadotropin and human placental lactogen, compounds that maintain maternal production of steroid hormones and regulate use of fetal glucose much like human growth hormone.[6] Finally, the placenta produces large amounts of lactate that is used by the fetus for gluconeogenesis.[7] Alterations in any of these and other placental functions may

lead to a compromised fetus such as a growth-retarded fetus.

As an initial step toward the study of placental metabolism by nuclear magnetic resonance spectroscopic methods, we used perchloric acid extract methods to characterize the phosphorus metabolites in term human placentas by ^{31}P magnetic resonance spectroscopy. In the studies described in this article we focused on the high-energy phosphate signals from the nucleoside triphosphates, in particular adenosine triphosphate. Alterations in the adenosine triphosphate levels and other adenosine nucleotides may be associated with pathologic conditions such as preeclampsia.[8, 9] In addition, high levels of placental adenosine triphosphate have been associated with high glucose levels in vitro[10] and may play a role in diabetic pregnancies, which often result in abnormally large infants. Lastly, ion transport, an important process in the placenta, is thought to be adenosine triphosphate–dependent because the addition of ouabain, an inhibitor of adenine triphosphatase, has been shown to affect such transport.[11] Therefore, abnormalities in placental metabolism may be shown by altered levels of adenosine triphosphate. Here we report results of our current efforts in the development of quantitation methods for adenosine triphosphate resonances in fresh and frozen human placental samples so that ultimately comparisons can be made between normal and abnormal placentas that may lend further insights into pregnancies that result in abnormal outcomes.

Material and methods

Fresh tissue preparation. Freshly delivered placental tissue from both vaginal and cesarean section births were studied. Approximately 5 gm of tissue was sharply dissected free from the remainder of the placenta and placed into a 20 mm nuclear magnetic resonance tube within 1 minute of delivery and maintained on ice for no more than 1 hour before spectra were acquired. Spectra were obtained with a 65° flip angle (40 μsec), an acquisition period of 1.12 seconds, a relaxation delay of 1 second (total pulse interval 2.12 seconds), and 256 acquisitions. These conditions were used to qualitatively assess ^{31}P spectral features and correspond to partially saturated inorganic phosphate resonances but to fully relaxed adenosine triphosphate signals inasmuch as the measured longitudinal (T1) relaxation value that determines the degree of relaxation for adenosine triphosphate in the tissue at 300 MHz field strength is 0.5 to 0.6 sec.

Tissue extracts. Perchloric acid extracts of placental tissue were prepared with a modification of a previously described technique.[12] Placentas were harvested by removing small pieces of tissue from the maternal side and freezing them in liquid nitrogen within 1 minute of delivery. Tissue trauma was minimized by use of sharp dissecting instruments, by removal of each piece with minimal dissection, and by performance of the dissection with the placenta remaining in the dissecting pan. The frozen tissue was pulverized in a custom-designed stainless steel mortar and pestle chilled with liquid nitrogen. A 20 gm sample of the finely ground tissue was mixed with 20 ml of 1 mol/L perchloric acid that had been precooled to the temperature of dry ice. The perchloric acid–tissue mixture was allowed to warm to −10° C in the ambient air for 15 minutes during which time the metabolites were extracted. It was then centrifuged at 40,000 rpm at −4° C for 20 minutes to separate the remaining solid fraction. The supernatant containing the extracted metabolites was decanted into a chilled beaker and neutralized to just above pH 7.0 through the dropwise addition of potassium hydroxide. The potassium perchlorate precipitated during neutralization was removed by a second centrifugation step identical to the first, and the supernatant was then passed over a chelating resin (Sigma Chemical Co., St. Louis) to remove divalent cations. The extract was again titrated to neutral pH with dilute perchloric acid, frozen in a vacuum flask (Virtis Co., Gardiner, N.Y.), and lyophilized to dryness. The extract was then resuspended in 5 ml of 40% deuterated water. For quantitation of spectra, the extracts were placed in the outer chamber of a coaxial 10 mm nuclear magnetic resonance tube with an external reference standard of 25 mmol/L hexachlorocyclotriphosphazene (Aldrich Chemical Inc., Milwaukee)[13] in the inner chamber. These spectra were obtained with a 70° pulse angle (21 μsec), an acquisition period of 1.12 seconds, a relaxation delay of 15 seconds (total pulse interval 16.12 seconds) and 160 acquisitions. These conditions yield fully relaxed adenosine triphosphate signals from the extract, inasmuch as the T1 relaxation time was measured to be approximately 1.8 seconds for the adenosine triphosphate signals of the extract.

Contribution from blood. To determine the contribution of blood to the tissue extract, we performed perchloric acid extracts of maternal blood (peripheral) and fetal blood (cord) from one patient. Perchloric acid (1 mol/L) was added in a 1:1 ratio to 20 ml blood and extracted according to the above protocol.

Nuclear magnetic resonance instrumentation. All spectra were obtained on a spectrometer (model GN-300WB General Electric, Fremont, Calif.) interfaced with a wide-bore superconducting magnet (89 mm bore, 7.0 tesla field) in the Duke Magnetic Resonance Spectroscopy Center. A standard 20 mm broad band probe (General Electric) tuned to 121.4 MHz was used for ^{31}P spectroscopy. The 90° pulses were: 56 μsec for a 20 mm tube with placental tissue; 27 μsec for a

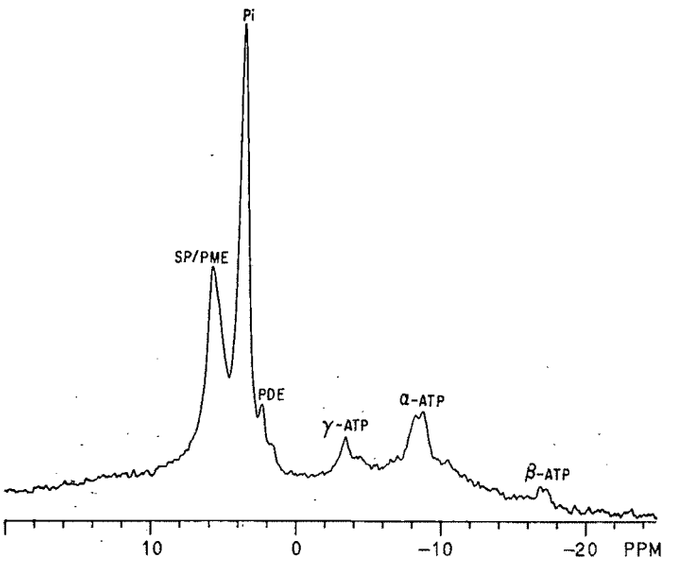


Fig. 1. 121 MHz ^{31}P magnetic resonance spectroscopy spectrum of gross placental tissue. Peaks identified are sugar phosphate–phosphomonoester, *SP/PME;* inorganic phosphate, *Pi;* phosphodiester, *PDE;* α-, β-, and γ-adenosine triphosphate. Spectrum obtained with 65° flip angle (40 μsec), acquisition period of 1.12 seconds, relaxation delay of 1 second (total pulse interval, 2.12 seconds), and 256 acquisitions.

10 mm tube with hexachlorocyclotriphosphazene–extract; 25 μsec for a 10 mm tube with hexachlorocyclotriphosphazene–adenosine triphosphate. Spectroscopic data were collected at the ambient operating probe temperature (approximately 22° C) without temperature regulation. To avoid radio frequency heating effects on the tissue and extracts, no proton decoupling was used. ^{31}P chemical shifts are referenced to phosphocreatine at 0.00 ppm.

External reference standard. Quantitation of adenosine triphosphate levels in the perchloric acid extracts was performed with the external reference standard of hexachlorocyclotriphosphazene and chromium acetylacetonate (Aldrich Chemical Inc.) in a coaxial capillary system.[13] Hexachlorocyclotriphosphazene has proved to be a useful ^{31}P chemical shift reference because its ^{31}P signal (approximately 24.4 ppm at our conditions) exhibits only a small shift with temperature (0.34 ppm between 8° and 50° C) and the narrow hexachlorocyclotriphosphazene signal is easily observed without proton decoupling. The T1 relaxation time of 25 mmol/L hexachlorocyclotriphosphazene in deuterated benzene at 121 MHz was 15 seconds. Addition of 31 mmol/L chromium acetylacetonate reduced the relaxation time for the hexachlorocyclotriphosphazene resonance to <200 msec. A calibration curve was constructed by recording nuclear magnetic resonance spectra for the two-compartment coaxial 10 mm nuclear magnetic resonance tube with 25 mmol/L hexachlorocyclotriphosphazene with 31 mmol/L chromium acetylacetonate in the inner chamber of a 10 mm coaxial insert and varying adenosine triphosphate (Sigma Chemical Inc., St. Louis) concentrations in the outer chamber. Spectra were acquired under identical conditions to those for the perchloric acid extracts. Peak area integration of the fully relaxed adenosine triphosphate and hexachlorocyclotriphosphazene signals generated the calibration curves. The ordinate is presented as the ratio of the area of the β-adenosine triphosphate resonance to that of hexachlorocyclotriphosphazene, whereas the abscissa represents the adenosine triphosphate concentration. This calibration curve gives a quantitative estimate of the concentrations of the various metabolites in the perchloric acid extracts. It is also used to estimate concentrations from peak areas in intact placental tissue although it must be recognized the signal attributed to β-adenosine triphosphate in the typical spectrum of whole tissue may include small contributions from other nucleoside triphosphates.[14] All adenosine triphosphate concentrations reported in this work are expressed as micromoles per gram of wet weight placental tissue.

Internal reference standard. To assess the reliability of quantitation with the external reference standard in our coaxial nuclear magnetic resonance tube, we compared results with those obtained by an internal standard. Because our spectra show at most very small signals from phosphocreatine in placental extracts, we chose to use it as the internal reference standard by spiking our perchloric acid extracts in a fixed volume

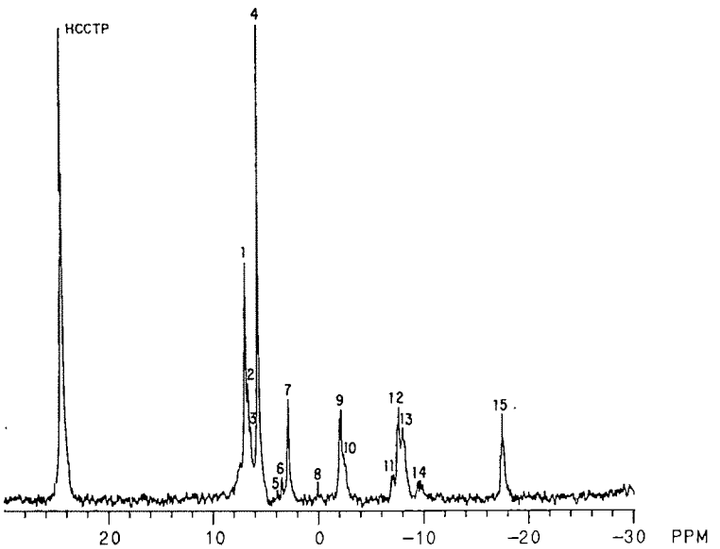


Fig. 2. 121 MHz ^{31}P magnetic resonance spectroscopy spectrum of perchloric acid extract of human placental tissue. Spectrum acquired with 70° flip angle (21 μsec), acquisition period of 1.12 seconds, relaxation delay of 15 seconds (total pulse interval, 16.12 seconds), and 160 acquisitions. Tentative peak assignments on the basis of literature: *1*, sugar phosphates, *2*, 2,3-diphosphoglycerate; *3*, phosphorylcholine; *4*, inorganic phosphate; *5*, glycerophosphorylserine; *6*, glycerophosphorylethanolamine; *7*, glycerophosphorylcholine; *8*, phosphocreatine; *9*, α-adenosine triphosphate; *10*, β-adenosine diphosphate; *11*, α-adenosine diphosphate; *12*, α-adenosine triphosphate; *13*, the oxidized form of nicotinamide-adenine dinucleotide; *14*, uridinediphosphate glucose; *15*, β-adenosine triphosphate. External reference standard, hexachlorocyclotriphosphazene, is at approximately 24.4 ppm with phosphocreatine at zero.

with a standard aliquot of 5 mmol phosphocreatine. Adenosine triphosphate concentrations were then calculated by comparison of the integrated peak areas with that of the added phosphocreatine.

Statistics. A linear regression analysis and a Pearson's correlation coefficient determination was performed to correlate the adenosine triphosphate levels determined with the use of the external reference standard with that found by the internal spiking method.

Results

A typical spectrum obtained from our preliminary studies of fresh placental tissue is shown in Fig. 1. The α-, β-, and γ-nucleoside triphosphates peaks are seen with the largest contributions to these resonances coming from adenosine triphosphate.[14] There is a large inorganic phosphorus peak, and significant resonances corresponding to phosphodiesters and to a sugar phosphate–phosphomonoester envelope. No identifiable resonance from phosphocreatine is observed in this spectrum, a result similar to that reported previously.[3]

Further spectral resolution of components in placental tissue is revealed in the corresponding spectrum of a perchloric acid placental extract in coaxial configuration with the external hexachlorocyclotriphosphazene reference standard as illustrated in Fig. 2. Resonances from specific phosphomonoesters and phosphodiesters such as 2,3-diphosphoglycerate, phosphorylcholine, serine phosphorylethanolamine, glycerophosphorylethanolamine, and glycerophosphorylcholine are clearly distinguishable. Peak assignments are made on the basis of previously reported identification of these compounds.[14] Even with the significantly higher signal/noise ratio and better resolution available in the extracts, only a small peak corresponding to phosphocreatine at 0.00 ppm is observed. Hexachlorocyclotriphosphazene appears as a single line at approximately 24.4 ppm downfield from the phosphocreatine position (0.00 ppm).

The calibration curve relating concentrations to relative nuclear magnetic resonance intensities determined for quantitating these studies is shown in Fig. 3. These data were obtained with commercially available adenosine triphosphate and hexachlorocyclotriphosphazene in a coaxial configuration identical to that used to study the placental extracts. Adenosine triphosphate concentrations from 11 human placentas were determined with both the external hexachlorocyclotriphosphazene standard and the internal spiking with added phosphocreatine. The measured values are listed in Table I and range from 0.404 to 0.709 μmol per gram.

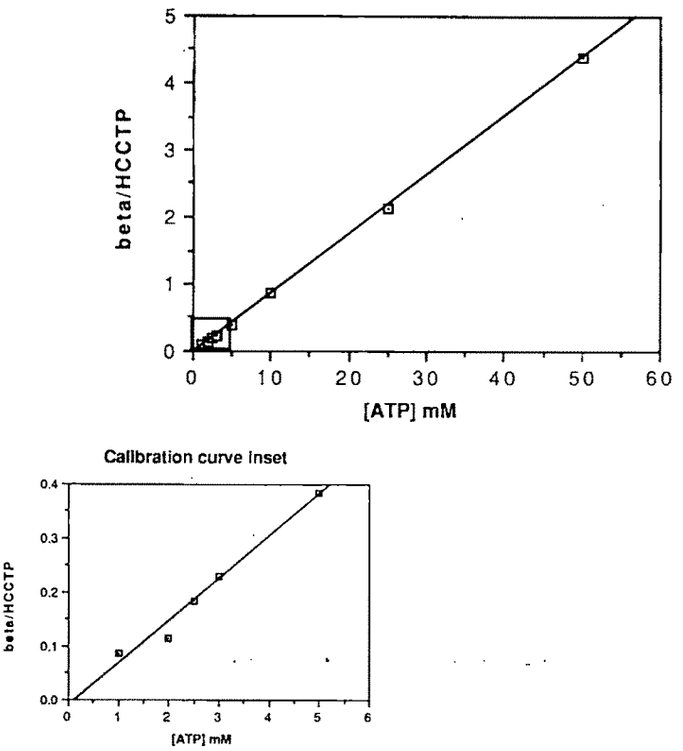


Fig. 3. Calibration curve for use of external reference standard with constant concentration of hexachlorocyclotriphosphazene and varying concentrations of adenosine triphosphate. *Inset* is adenosine triphosphate at lower concentrations.

The correlation coefficient of comparisons between the two methods is 0.755.

Fig. 4 shows a ^{31}P spectrum from maternal blood. The spectrum from fetal cord blood is essentially identical. The significant peaks are those from adenosine triphosphate, 2,3-diphosphoglycerate, and inorganic phosphate. The adenosine triphosphate calculated from the maternal peripheral blood is 0.256 μmol per milliliter blood. Because each gram of placenta represents roughly 1 ml in volume, the adenosine triphosphate contribution from blood in this placenta can be estimated to be approximately 0.205 μmol per gram tissue, assuming that approximately 80% of the placenta is composed of blood (maternal and fetal).[15] This result suggests that blood with this concentration of adenosine triphosphate may actually account for as much as 30% to 50% of the total adenosine triphosphate seen in the tissue extracts and presumably a similar contribution would be observed in in vivo or ex vivo spectra of whole placental tissue.

Comment

Although magnetic resonance spectroscopy has been applied to other tissues and tissue extracts, its use is only beginning to be explored for studies of tissue in the female reproductive tract. Earlier work by Noyszewski et al.[3] has focused on ^{31}P spectroscopy of gross placental tissue that showed resonances for sugar phosphate or phosphomonoester, inorganic phosphate, and adenosine triphosphate but no visible phosphocreatine. Before our systematic efforts at more detailed characterization and quantitation of metabolites, we also examined fresh tissue samples and obtained similar results as illustrated in Fig. 1.

The overall purpose of this work was to explore the applicability of the perchloric acid extraction technique for quantitative investigations of metabolites in placental tissue and to establish its correlation with gross placental tissue. As can be seen in Fig. 2, the spectrum from an extract gives significant peak enhancement and resolution and indeed allows observation of very small quantities of metabolic substrates such as phosphocreatine that are not easily observed in gross tissue. These extract spectra show that those from placentas are different from those seen in the heart,[16] which has relatively high concentrations of adenosine triphosphate and phosphocreatine, but are similar to those seen in liver, which has low concentrations of phosphocreatine.[17] This low phosphocreatine concentration found in placenta appears to be an intrinsic property

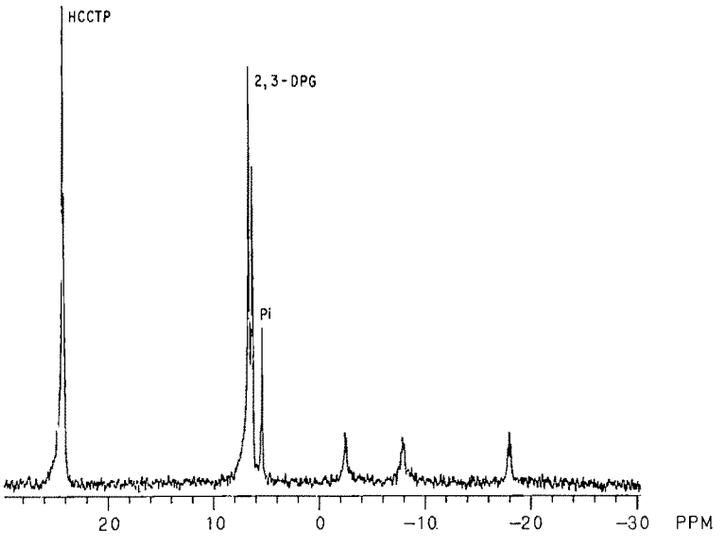


Fig. 4. 121 MHz ^{31}P spectrum from perchloric acid extract of maternal blood. Fetal blood showed identical spectrum. External reference standard: hexachlorocyclotriphosphazene, *HCCTP;* 2,3-diphosphoglycerate, *2,3 DPG;* inorganic phosphate, *Pi.*

Table I. Adenosine triphosphate quantitation in placental extracts

Sample	*Hexachlorocyclotriphosphazene standard (μmol/gm wet weight)**	*Phosphocreatine standard (μmol/gm wet weight)†*
1	0.404	0.554
2	0.423	0.496
3	0.433	0.515
4	0.445	0.747
5	0.463	0.486
6	0.521	0.677
7	0.576	0.683
8	0.608	0.675
9	0.625	0.639
10	0.690	0.995
11	0.709	0.791

*Mean ± SD, 0.536 ± 0.111.
†Mean ± SD, 0.659 ± 0.151.

of this tissue, suggesting that other energy metabolism pathways aside from those driven by creatine kinase may also be significant.

In the performance of our spectral analysis, we noted that there were large amounts of inorganic phosphate in placental tissue. Quantitation with the same method described for quantitation of adenosine triphosphate yielded levels that ranged from 2.045 to 15.575 μmol per gram wet weight. Corrections were made for the inorganic phosphate resonances being only 97.4% relaxed under our spectral conditions. The levels calculated are significantly higher than the adenosine triphosphate levels (range, 0.404 to 0.709 μmol per gram). This is especially interesting because there are low levels of phosphocreatine and adenosine triphosphate. This large amount of inorganic phosphate most likely did not originate from phosphocreatine or adenosine triphosphate breakdown. It also does not appear that this is an indirect contribution from blood in the tissue inasmuch as our calculated concentration of inorganic phosphate is 0.647 μmol per milliliter of blood, or only 0.518 μmol per gram tissue. This suggests that inorganic phosphate in placenta may play a major role in other phosphorus metabolic processes aside from oxidative phosphorylation. Iles et al.[18] suggested that the inorganic phosphate seen in rat liver extracts may derive from labile organic phosphates that liberate inorganic phosphate on extraction or that the inorganic phosphate may exist in an available pool for metabolic processes. Inorganic phosphate in placenta probably

behaves in a similar fashion. Because glycolysis is an important process in the placenta, a large amount of inorganic phosphate may be needed to stimulate enzymes such as glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglycerate kinase, which are important enzymes in glycolysis. Perhaps future studies of inorganic phosphate levels in normal and abnormal placentas will prove to give informative biomedical insights.

The prominent peaks in the phosphomonoester and phosphodiester regions of the spectra should also be pointed out and may signify enhanced membrane synthesis that has previously been noted in spectra of tumors.[19] In many ways the placenta has been likened to a large, rapidly growing tumor.

One other aim in this study was to perform quantitation of phosphorus metabolites in our tissue extracts with a reference standard. Use of either external or internal intensity standards in our extracts allows quantitation of adenosine triphosphate levels and thus meaningful comparative analysis between different placentas. We are interested in the development of these methods because such analysis may allow metabolic studies of normal and abnormal placentas, which can be used to evaluate abnormal conditions such as those associated with growth-retarded infants. The levels of adenosine triphosphate identified in normal and abnormal placentas may ultimately be applied to in vivo placental studies. Clearly the absence of quantitation has presented a problem in many previous comparative nuclear magnetic resonance studies. The adenosine triphosphate concentrations measured here for human placental tissue fall in the range of 0.40 to 0.70 μmol per gram wet weight on the basis of a hexachlorocyclotriphosphazene standard. Whereas this is low compared with some other types of tissues, it agrees with published values from other placental studies that were in the range of 0.30 to 0.70 μmol per gram wet weight[20] as determined by spectrophotometric analysis and high-performance liquid chromatography on freeze-clamped tissue.

The external coaxial capillary method with hexachlorocyclotriphosphazene has the advantage that it is portable and can be used reproducibly both in extracts and in perfused tissue samples. Use of an external standard rather than an internal standard would minimize variability in extraction rates, a difficulty that can be encountered in perchloric acid extractions. Reproducibility of the current extraction and quantitation technique was tested with repetitive measurements on several tissue samples from the same placenta with essentially identical results.

The contribution of blood to the total adenosine triphosphate measured is an important consideration in studies of highly vascular organs such as kidney, liver, and vascular tumors that may not be commonly appreciated. Our calculations indicate that the blood component may account for as much as 30% to 50% of the total adenosine triphosphate in our samples. Therefore, it appears important to make corrections for the adenosine triphosphate component from blood when measuring the total adenosine triphosphate levels in vascular tissues. This by necessity will depend on the approximate blood volume present and an estimate of blood adenosine triphosphate levels for a representative blood sample. A correction of this type will be important not only in perchloric acid extracts but also in in vivo spectroscopy studies.

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Argon laser versus microscissors for hysteroscopic incision of uterine septa

Giovanni Battista Candiani, MD, Paolo Vercellini, MD, Luigi Fedele, MD, Salvatore Garsia, MD, Diana Brioschi, MD, and Laura Villa, MD

Milan, Italy

We performed hysteroscopic metroplasty in 21 women with repeated abortion and subseptate uterus. The patients were randomly allocated to septal incision with the argon laser (group I, 10 subjects) or microscissors (group II, 11 subjects) to compare these instruments in terms of surgical feasibility and anatomic results. The mean operating time was 57% longer in group I than in group II (p = 0.001), the intra- and postoperative morbidity of the whole series was negligible, and the anatomic results at abdominal ultrasonography and hysteroscopy performed 2 months postoperatively were similar in the two groups. This study confirms that microscissors are the simplest, fastest, most effective, and least expensive instrument to correct a septate uterus. The complete agreement of the findings at follow-up hysteroscopy and ultrasonography suggest the use of the latter as the method of choice to check the surgical results. (Am J Obstet Gynecol 1991;164:87-90.)

Key words: Uterine malformations, recurrent abortion, hysteroscopy, laser

Hysteroscopic incision seems to be the method of choice to correct a septate uterus.[1-3] The endoscopic use of microscissors and the resectoscope has proved successful,[1-4] and hysteroscopic metroplasty with fiberoptic laser has been proposed.[5] We designed a randomized study to compare the surgical and anatomic results of section of the uterine septum performed with the argon laser versus microscissors.

Material and methods

During 1989 we studied 21 women with a mean age of 28 years (range, 23 to 34) with two or more spontaneous abortions, a double uterine cavity at hysterosalpingography (Fig. 1) and evidence of a normal uterine fundus at ultrasonographic examination with a half-full bladder.[6, 7] All the uteri were classified as American Fertility Society class Vb (partial septate uterus).[8]

After giving informed consent, the patients were allocated according to a randomization list to hysteroscopic metroplasty with argon laser (group I, 10 women) or microscissors (group II, 11 women) between day 7 and day 10 of the cycle. Preoperative hysterosalpingography and ultrasonography showed the septum was broad (>3 cm wide at the base) in two subjects in group I and in four subjects in group II. Under general endotracheal anesthesia and laparoscopic control, the cervix was dilated to 7 mm and a rigid hysteroscope (model 26157 B, Storz Endoscopy, Tuttlingen, Federal Republic of Germany) with a 7 mm diameter operating sheath (Storz model 26163 C and 26163 H) was introduced. The uterine cavity was distended with a 10% solution of dextran of molecular weight 40,000 in normal saline solution (Rheomacrodex, Baxter-Travenol, Trieste, Italy). In the group I women the septal incision was performed with the argon laser (model 20, HGM Medical Laser Systems Inc., Salt Lake City, Utah), passing the 0.6 mm flexible quartz fiber through the operating channel of the hystero-

From the First Department of Obstetrics and Gynecology, University of Milano School of Medicine.
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Reprint requests: Paolo Vercellini, MD, First Department of Obstetrics and Gynecology "L. Mangiagalli," University of Milano School of Medicine, Via Commenda 12, Milano, Italy 20122.
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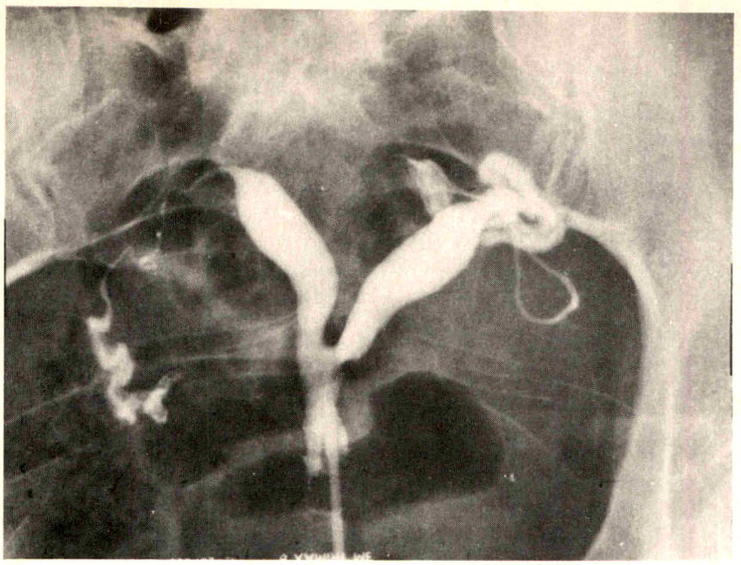


Fig. 1. Hysterosalpingogram of septate uterus before hysteroscopic metroplasty with argon laser.

scopic sheath and using a touch technique. The power was set at 12 watts in continuous mode. Rigid scissors (Storz model 26158 EK) were used in the group II patients.

After visualization of the tubal ostia, the section was started from the inferior margin of the septum and carried cephalad with progressive horizontal incisions in the midline. The six broad septa were sectioned with the technique suggested by March and Israel.[2] The incision was considered complete when a normal uterine cavity was obtained and the hysteroscope could be moved freely from one tubal ostium to the other. The operating time was calculated from the insertion of the flexible fiber or microscissors into the uterine cavity to the removal of the hysteroscope. No intrauterine contraceptive device was inserted and no pre- or postoperative hormone treatment was administered. The patients were scheduled to undergo transabdominal ultrasonography in the late secretory phase of the second postoperative cycle and follow-up hysteroscopy in the next proliferative phase. The ultrasonographist did not know the surgical results and the hysteroscopist was unaware of the ultrasonographic findings. For statistical analysis the Student *t* test and Fischer's test were used.

Results

All the procedures were completed without complications. Incision of the septum with argon laser was relatively slow and difficult. The mean operating time was 57% longer than with microscissors, the difference being 11 minutes (95% confidence limits, 5 to 17; Table I). This was attributable partly to the lower cutting capability of the laser fiber and partly to the difficult operating conditions and to the reduction of the visual field by the eye protection filter. The mean amount of distension medium used was 75% greater with the argon laser, with a difference of 333 ml (95% confidence limits, 105 to 561). The postoperative course was uneventful in all patients, and they were discharged within 24 hours.

At follow-up ultrasonographic examination a residual fundal notch ≥1 cm deep with respect to the orthogonal plane passing through the two tubal ostia was observed in two women in each group. Minor fundal irregularities (<1 cm deep) were present in four group I and five group II patients. The hysteroscopic findings always confirmed the ultrasonographic data. All the uterine cavities had a uniform lining of grossly normal endometrium. During the repeat hysteroscopy the four central fundal adhesions of ≥1 cm deep were sectioned. Three group I and two group II subjects had a follow-up hysterosalpingography that confirmed the ultrasonographic and hysteroscopic findings (Fig. 2).

Six patients who underwent surgery with the argon laser attempted to conceive: one aborted at 10 weeks' gestation, one took a pregnancy to term and was delivered vaginally, three are currently pregnant (14, 19, and 28 weeks, respectively), and one has not conceived after 6 months. Of the eight women who underwent surgery with microscissors who sought a pregnancy, one underwent right salpingectomy for ectopic pregnancy, one had a vaginal delivery at term, one had a cesarean section at 39 weeks' gestation for obstetric reasons, four have an ongoing pregnancy (7, 9, 18, and 26 weeks), and one has not conceived after 4 months.

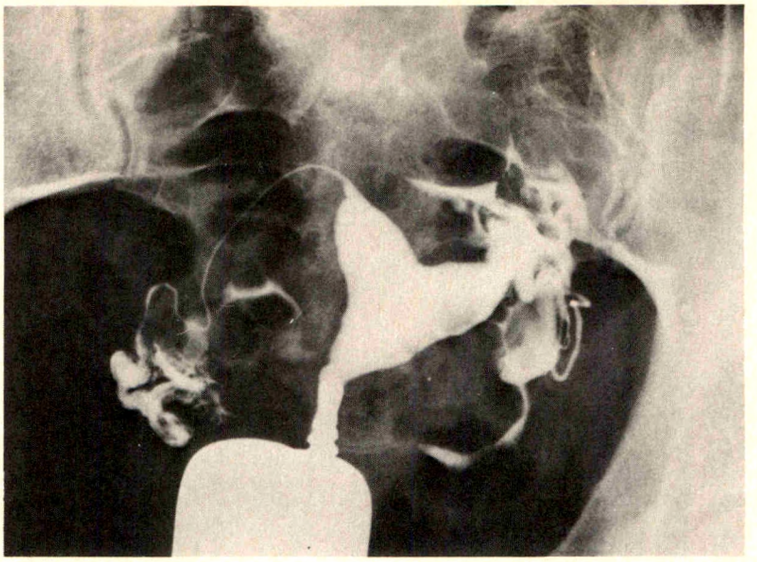


Fig. 2. Same case as in Fig. 1. Postoperative hysterosalpingogram shows minimal septal remnant <1 cm deep.

Comment

The argon laser is a simple instrument to use, and is practical and effective in the laparoscopic treatment of endometriosis.[9] Energy is delivered through a flexible quartz optical fiber of 0.3 or 0.6 mm diameter. Unlike the carbon dioxide laser, the argon laser can be used through clear fluids, and is thus suitable for operative hysteroscopy. Also the Nd-YAG laser can be used with fibers through fluids, but because of the power of its beam, which can penetrate to depths of 4 to 5 mm and thus effectively ablate endometrium,[10] it does not seem to be indicated in conservative intrauterine surgery where endometrial sparing is most important. The argon laser can coagulate small vessels and, when used at relatively high power settings with a near-contact or touch technique, the predominant effect is vaporization and incision with an extremely narrow band of thermal damage to surrounding tissues.[9] Because of these characteristics we considered the argon laser (the KTP/532 laser is not yet available in Italy) the best choice for a comparative study on microscissors, the current "gold standard" for hysteroscopic metroplasty.[1-3]

Our study had a major limitation, the small sample size, but it was designed as a pilot study to identify clinically important differences in the feasibility and duration of operation and in the postoperative anatomic results. This initial experience of hysteroscopic correction of septate uteri with the argon laser was unsatisfactory. The operating times were appreciably longer than when microscissors were used. The slowness of the incision could possibly be related to the range of light wavelength of the argon laser (488 to 514 mm), which is preferentially absorbed by tissues rich in red pigment such as hemoglobin, whereas the cut septum is white and avascular. When the section reaches the cranial part of the septum, arteriolar bleeding is frequently observed. The argon laser's power of coagulation seems of no advantage in these circumstances. In fact, as noted by others, bleeding from the incised septum is minimal: fundal bleeding is the physiologic sign that parietal myometrium has been reached.[1-3]

Table I. Data on hysteroscopic incision of uterine septa with argon laser or microscissors

	Group I, argon laser (n = 10)	*Group II, microscissors (n = 11)*
Operating time (min)	30 ± 7*	19 ± 6†
Range	16-41	12-37
Distension medium used (ml)	776 ± 228*	442 ± 266 ml‡
Range	340-1280	240-1150
No. of patients (%) with residual fundal notch ≥ 1 cm deep at follow-up ultrasonography and hysteroscopy	2 (20%)	2 (18%)

*Mean ± SD.
†$p = 0.001$ versus group I (Student's t test).
‡$p = 0.006$ versus group I (Student's t test).

After the section we did not insert an intrauterine contraceptive device nor did we administer estrogens inasmuch as in a previous study we had found these measures of no benefit.[11] Postoperative morbidity was negligible in both groups. We observed no differences in the intracavitary morphologic results after metroplasty with the argon laser compared with microscissors. The follow-up of the patients is too short to draw conclusions on the reproductive outcome.

As a secondary observation we found that the data obtained at the follow-up ultrasonographic examination corresponded to those of the "second look" hysteroscopy. If this finding is confirmed, it would mean that an ultrasonographic scan with half-full bladder[6, 7] would be sufficient to check the results of surgery before allowing patients to try to conceive. Hysteroscopy could be reserved for patients who require correction of deep septal remnants.

This study confirmed that microscissors are the most simple, rapid, effective, and economic instrument for hysteroscopic correction of a septate uterus. Other disadvantages of the argon laser include the necessity for running water to cool the laser generator and for a special three-phase electricity supply. The eye safety filter necessary to protect the operator's retina from backscatter alters color perception, reduces the field of view, adds an extra encumbrance to the eyepiece of the hysteroscope, and makes photographic or television documentation of the operation difficult. It takes longer to learn to use the argon laser than microscissors in hysteroscopy. Lastly, an argon laser has a very high initial cost and should be acquired only by hospitals that can amortize the expense on a large number of endoscopic operations. Otherwise the economic advantage of hysteroscopic metroplasty[3] is lost.

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Angiosarcoma of the uterus: A case report

Guillermo E. Quinonez, MD, MSc, Maria P. Paraskevas, MD, MSc, Malkit S. Diocee, EMT, and Susan M. Lorimer, MD

Winnipeg, Manitoba, Canada

We are reporting a case of angiosarcoma of the uterus in which the diagnosis was confirmed ultrastructurally by demonstration of Weibel-Palade bodies in the tumor cells. Only 10 cases of this entity have been previously documented in the literature. (Am J Obstet Gynecol 1991;164:90-2.)

Key words: Uterine angiosarcoma, Weibel-Palade bodies

From the Department of Pathology, Health Sciences Centre, Faculty of Medicine, University of Manitoba.
Received for publication July 30, 1990; accepted August 8, 1990.
Reprint requests: G. Quinonez, MD, 820 Sherbrook St., Winnipeg, Manitoba, Canada R3A 1R9.
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Angiosarcomas of the uterus are rare. Ten cases have been previously documented in the literature.[1, 2] We are reporting a case of angiosarcoma of the uterus in which the diagnosis was confirmed ultrastructurally by demonstrating Weibel-Palade bodies.

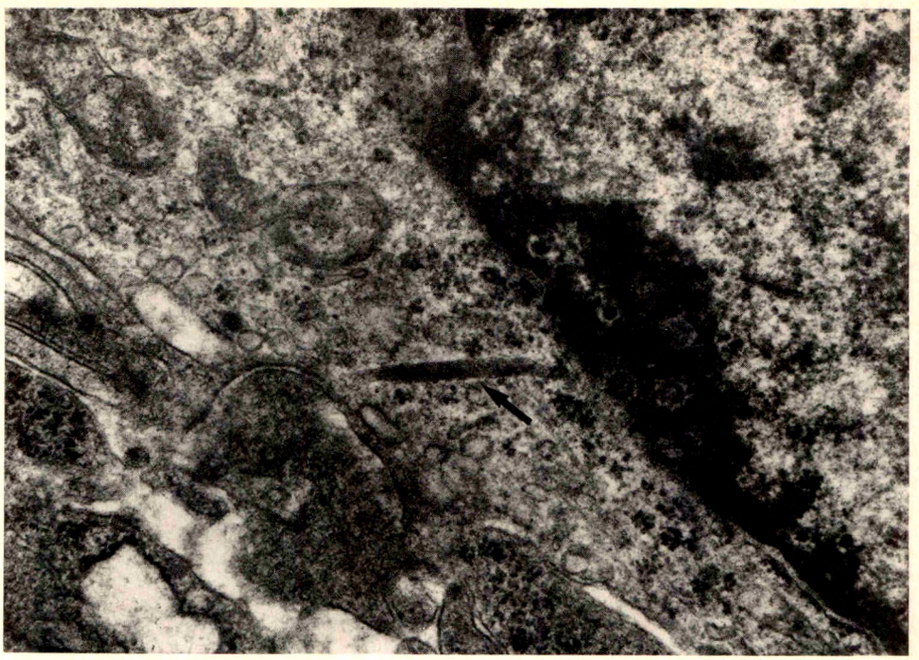


Fig. 1. Weibel-Palade body is seen at center of figure *(arrow)*. Micropinocytotic vesicles and long cytoplasmic extensions from neighboring cells are also seen. Intermediate junctions are present between those extensions (center, upper part). (×36,000.)

Case report

A 65-year-old woman presented to her family physician in April 1985 with postmenopausal bleeding. A curettage was performed, and findings were interpreted as angiosarcoma. Results of endocervical curettage were noncontributory.

Gynecologic history included menarche at age 14 and normal regular menses. She was gravida 3, para 3 and had uneventful vaginal deliveries. She had had two previous curettages because of perimenopausal bleeding in 1967 and postmenopausal bleeding in 1982. Results of both curettages were reported as unremarkable.

Two months before this hospital admission, she noted increasing fatigue and a 10-pound weight loss over the last 2 weeks. A general physical examination showed her condition to be essentially normal. Pelvic examination showed a brownish red discharge from the endocervix. The uterus was bulky and measured approximately 7.5 cm in length. Adnexal and rectal examinations gave negative results. Findings on intravenous pyelography and barium enema were negative. The hemoglobin level was 116 mg/L, white blood cell count was 8000/ml, and platelet count was 298,000/ml. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and selective pelvic lymphadenectomy followed by vaginal insertion of cesium and nine courses of combination chemotherapy with cisplatin and Adriamycin. At the last follow-up visit in March 1989, the patient was clinically free of disease. A vaginal smear showed no abnormal cells and the chest x-ray film did not show any abnormalities.

Tissue received at the laboratory consisted of uterus with adnexa, right and left pelvic nodes, and omental biopsy specimen. The uterus weighed 100 gm. The endometrial cavity measured 3.5 × 2.4 cm. Arising from the anterior aspect of the uterus, toward the fundus, was a large hemorrhagic mass measuring 4.7 × 4 cm and raised 1.9 cm above the endometrial layer. On section, this mass had a gray hemorrhagic appearance and a finely lobulated pattern. An additional satellite nodule measuring 0.9 cm in diameter was present posteriorly at the fundus, toward the right cornu. Tubes and ovaries were atrophic and otherwise unremarkable.

Microscopic sections of the hysterectomy specimen showed an endometrial tumor similar to the reviewed curettage specimen. It consisted mostly of solid masses of pleomorphic and spindle cells forming communicating channels filled with blood. The nuclei were irregular and hyperchromatic with occasional nucleoli, and the cytoplasm was eosinophilic with no obvious cytoplasmic borders; mitoses were up to 6 per 10 high-power fields. True blood vessels were not found within the tumor. Reticulin stain showed abundant reticulum network outlining interconnecting spaces. The tumor infiltrated the myometrium irregularly and extended to half its thickness. The satellite nodule showed similar histologic features. The remaining endometrium showed only cystic and atrophic glands. Pelvic nodes and omentum were free of tumor.

Ultrastructurally, cells lining the vascular spaces showed micropinocytotic vesicles and, not infrequently, Weibel-Palade bodies (Fig. 1). Factor VIII–related antigen immunocytochemical stain was negative in the tumor cells.

Comment

The present case was that of a 65-year-old woman presenting with postmenopausal bleeding who underwent surgical treatment and chemotherapy after diagnosis of angiosarcoma of the uterus. She was doing well 4 years after operation. This clinical history is similar to some of the cases previously reported.[1, 2]

This case showed, by light microscopy, typical features of a vascular tumor, the pattern of which was well demonstrated by reticulin stain. The true vascular nature of the tumor was confirmed by demonstrating Weibel-Palade bodies in the tumor cells ultrastructurally. An immunocytochemical test for Factor VIII–related antigen gave negative results. A negative result of Factor VIII–related antigen staining does not rule out the diagnosis of angiosarcoma since Factor VIII–related antigen positivity is highly variable in cases of angiosarcoma (65% to 95%).

This report adds a new case of this extremely rare entity. It confirms ultrastructurally, for the first time in a published case, the vascular nature of the neoplasm by demonstrating Weibel-Palade bodies in the tumor cells.

We thank Dr. A. Alguacil-Garcia for his comments.

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Quantitative and qualitative platelet abnormalities during pregnancy

Helen Y. How, MD, Frauke Bergmann, MD, Mabel Koshy, MD, Juan Chediak, MD, Celeste Presperin, RN, and Stanley A. Gall, MD

Chicago, Illinois

In a 1-year period, 15 of 4048 pregnant women were found to have thrombocytopenia during their first prenatal visit. Their qualitative and quantitative platelet abnormalities were followed up prospectively for 1 to 6 months post partum. Platelet counts returned to normal in 14 of 15 patients 4 to 6 weeks post partum. von Willebrand factor antigen and ristocetin cofactor activity were low in three of 15 patients 4 to 6 weeks post partum, but had been normal during the antepartum period. Two of these three patients had prolonged bleeding times ante partum and post partum. All three patients subsequently were found to have mild type I von Willebrand disease. Six patients had detectable platelet antibodies. Neonatal thrombocytopenia was found in one term infant of a patient with mild thrombocytopenia and negative platelet antibodies. This study suggests that mild, transient, isolated thrombocytopenia can occur in an otherwise normal pregnancy and its incidence may be lower than previously reported. Extensive testing is not indicated unless there is an associated prolonged bleeding time. The possibility of von Willebrand disease or a qualitative platelet disorder should be considered. (Am J Obstet Gynecol 1991;164:92-8.)

Key words: Thrombocytopenia, pregnancy, von Willebrand disease

From the Department of Obstetrics and Gynecology and Division of Hematology/Department of Medicine, University of Illinois at Chicago, and Michael Reese Hospital.
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Received for publication March 30, 1990; revised August 10, 1990; accepted August 13, 1990.
Reprint requests: Helen How, MD, University of Louisville School of Medicine, Department of Obstetrics and Gynecology, Louisville, KY 40292.
6/1/24572

Mild, transient, isolated thrombocytopenia has been reported to occur during pregnancy with a frequency of 7.6% to 24%.[1, 2] Postulated causes of this transient mild thrombocytopenia include immune complex deposition on platelets during a viral infection with removal from circulation,[1] chronic intravascular coagulation during normal pregnancy,[3] and the "tail end" of a normal platelet distribution.[4] All re-

ported cases had uncomplicated perinatal and maternal outcomes[1-3]; however, none of the reports has evaluated the patients for the presence of qualitative platelet defects associated with the thrombocytopenia. The purpose of our prospective study was as follows: (1) determine the incidence of thrombocytopenia at the time of the first prenatal visit; (2) assess whether mild thrombocytopenia was associated with a qualitative platelet abnormality; (3) determine whether there was a correlation between thrombocytopenia and abnormal levels of von Willebrand factor; and (4) develop guidelines for appropriate management of this condition.

Material and methods

All pregnant women attending the obstetric outpatient clinic at the University of Illinois Hospital at Chicago from August 1987 to August 1988 were screened for thrombocytopenia at the time of their first prenatal visit. Thrombocytopenia during pregnancy is defined as a platelet count of $\leq 150{,}000/\mu l$.[5] Patients with thrombocytopenia associated with diagnosed collagen vascular disease, preeclampsia-eclampsia, drug ingestion, infection, hemoglobinopathies, immune thrombocytopenic purpura, and other systemic diseases were excluded. Only patients with newly identified thrombocytopenia without a known cause were included in the study.

After written informed consent was obtained, the study patients were followed up throughout the prenatal and postpartum periods. A detailed medical and obstetric history including evidence of a bleeding diathesis and nutritional status and a complete physical examination were obtained for all patients. Laboratory evaluation included a complete blood count with platelet count and mean platelet volume, bleeding time, antinuclear antibody, rheumatoid factor, prothrombin time, activated partial thromboplastin time, fibrinogen, fibrin split products, serum folate, vitamin B_{12}, platelet antibody, serum albumin levels, von Willebrand factor antigen (vWF:Ag) and ristocetin cofactor activity (vWF:RiCof) assays. The complete blood count with platelet count was repeated every 2 to 4 weeks as indicated until 4 to 6 weeks post partum. Bleeding time, vWF:Ag, vWF:RiCof, and platelet antibody measurements were repeated at 4 to 6 weeks post partum and again at 5 to 6 months post partum if necessary.

Complete blood count, platelet count, and mean platelet volume measurements were performed on venous blood anticoagulated with ethylenediaminetetraacetic acid (EDTA) with an automated counter (S-Plus IV model, Coulter Electronics, Hialeah, Fla.). The coefficient of variation for platelet count is 2.3%. The degree of thrombocytopenia was confirmed by examination of the stained peripheral blood smear to exclude the presence of platelet clumping and other hematologic disorders.

Modified Ivy bleeding time measurements were performed with a Simplate single blade automatic device (General Diagnostic, Morris Plaines, N.J.). The normal bleeding time ranges between 2 and 8 minutes.

Blood specimens for coagulation studies were collected in Vacutainers blood collecting tubes containing 3.8% sodium citrate in a ratio of 9:1 (blood/citrate). Platelet-poor plasma was obtained by centrifugation of the blood samples at 3300 *g* at 2° C. An aliquot of the plasma was stored in plastic tubes at −70° C until tested. Pooled plasma specimens from at least 20 normal control subjects (males and females) was used as a reference plasma for all procedures. Each test was performed twice and the average was reported. The quantification of vWF:Ag was performed with the Laurell immunoelectrophoresis[6] with an antibody (Behring Diagnostic, La Jolla, Calif.) to von Willebrand factor. The functional activity of von Willebrand factor was measured by use of a nephelometer (Chronolog Corp., Havertown, Pa.) in accordance with the procedure by Weiss[7] with the following modifications. Washed platelets (with artificial media) of a healthy control subject at a concentration of $380{,}000/\mu l$ were used. A 100% standard was obtained by use of pooled normal plasma; 0.12 ml of plasma and 0.4 ml of the platelet suspension were mixed in a cuvette with 0.02 ml of calcium chloride (1.5 mg/ml) at 37° C. Agglutination was induced by addition of 0.04 ml of ristocetin (22 mg/ml 0.9% normal saline solution). Because ristocetin is used as a ligand and this method checks the activity of von Willebrand factor, this test is known as the vWF:RiCof assay. To differentiate the various subtypes of von Willebrand disease, the multimeric distribution of von Willebrand factor was determined with a 1.4% low-temperature agarose gel (Type VII, Sigma Chemical Co., St. Louis) following a modified method of Ruggeri and Zimmerman.[8]

Unbound platelet antibodies were measured with the fluorescence activated flow cytometry method and results were reported as a reciprocal of a titer (Meth Path, Teterboro, N.J.).[9] Fibrinogen was determined with reagents purchased from Dade Diagnostic Inc. (Miami). Fibrin split products were measured with monoclonal antibody–coated latex particles (American Diagnostica Inc., New York). Prothrombin time and activated partial thromboplastin time were determined following the coag-A-mate operator's manual provided by General Diagnostic. Albumin was measured spectrophotometrically at 630 nm after binding to bromocresol green dye. Urine toxicology screen was performed by fluorescence polarization immunoassay with a therapeutic drug analyzer (Abbot, Irving, Tex.). The following drugs and their metabolites were checked: barbiturates, benzodiazepines, cocaine, opiates, phencyclidine, amphetamine, methamphetamine, and cannabinoids. Rheumatoid factor was determined by use of Behring's

Table I. Maternal characteristics

Patient no.	*Age (yr)*	*Race*	*Gravity*	*Parity*	*Associated findings*	*Gestational age at first prenatal visit (wk)*
1	38	H	8	2-0-5-1	Intravenous drug abuser ANA 1:160 speckled	5
2	37	H	9	7-7-1-7	Negative	33
3	34	H	6	3-0-2-3	FTA-positive RPR = 1:1	22
4	30	H	2	1-0-0-1	Negative	28
5	23	H	4	3-0-0-3	Negative	30
6	20	H	2	1-0-0-1	Negative	31
7	18	H	2	1-0-0-1	Negative	23
8	33	B	5	4-0-0-4	ANA 1:640 centromere	33
9	25	B	1	0	Negative	28
10	20	B	1	0	Negative	37
11	19	B	2	0-0-1-0	Negative	34
12	19	B	4	1-0-2-1	Cocaine and marijuana used	30
13	16	B	1	0	Negative	19
14	16	B	1	0	Negative	30
15	15	B	1	0	Negative	37

B, Black; *H*, Hispanic; *ANA*, antinuclear antibody; *FTA*, fluorescent treponemal antibody-absorbed test; *RPR*, rapid plasma reagin test.

Rapi Tex RF test (Sommerville, N.J.). Antinuclear antibody test was determined by staining the antigen-antibody complex formed in the presence of antinuclear antibody with fluorescein-labeled antihuman globulin (Antibodies Inc., Davis, Calif.).

Comparisons of platelet counts measured on the same persons during the course of their pregnancies were analyzed with a randomized, block design analysis of variance. Comparison of vWF:Ag and vWF:RiCof between normal pregnancies and pregnancies with subsequent diagnosis of von Willebrand disease were performed with the Student *t* test for independent samples.

Results

In our patient population, 15 of 4048 patients met the diagnostic criteria for transient thrombocytopenia. Eight other patients were excluded from the study: three with thrombocytopenia associated with collagen vascular disease; two with preeclampsia-eclampsia; one for drug ingestion; and two with immune thrombocytopenic purpura.

The mean maternal age was 24 years (range, 15 to 38). Five patients were primigravid and 10 were multiparous. Eight were black and seven were Hispanic (Table I). All patients denied the ingestion of medications known to affect platelet function.

Ten of the 15 patients had persistent antepartum thrombocytopenia in at least three consecutive determinations (mean platelet count ± SEM of 112,638 ± 2,877/μl). Of the remaining five patients who had their initial evaluation in the third trimester, three patients (Nos. 2, 6, and 12) had only one of their three serial platelet counts in the thrombocytopenic range (98,000, 121,000, and 125,000/μl, respectively); the other two had counts in the thrombocytopenic range in more than one sample. The mean platelet count ± SEM at initial diagnosis for the 15 patients during the antepartum period was 118,620 ± 5040/μl and was significantly lower than the mean of 193,460 ± 10,890/μl seen in the same patients at 4 to 6 weeks post partum ($p << 0.0001$). The platelet counts of 14 (93.3%) patients were normal at 4 to 6 weeks post partum. Only patient No. 5 remained slightly thrombocytopenic (146,000/μl), and mild type I von Willebrand disease was subsequently diagnosed. The mean platelet count for the 15 patients was 137,460 ± 18,190/μl at delivery and 133,150 ± 14,480/μl 1 to 5 days post partum (Fig. 1). These values were not significantly different from the antepartum values but were significantly different from the 4- to 6-week postpartum value ($p << 0.002$ and $<< 0.001$, respectively). The mean platelet volume did not change significantly throughout the prenatal, peripartum, and postpartum course.

Five of the 15 patients (33.3%) had a prolonged bleeding time (Table II). Type I von Willebrand disease was diagnosed on the basis of additional laboratory data in two of the patients (Nos. 5 and 7) who had persistently prolonged bleeding time (>15 minutes) during the postpartum period. The bleeding time in the remaining three patients returned to normal values in the postpartum period.

Six of the 15 patients (Nos. 2, 3, 5, 7, 8, and 13) had positive unbound platelet antibodies. One of these six

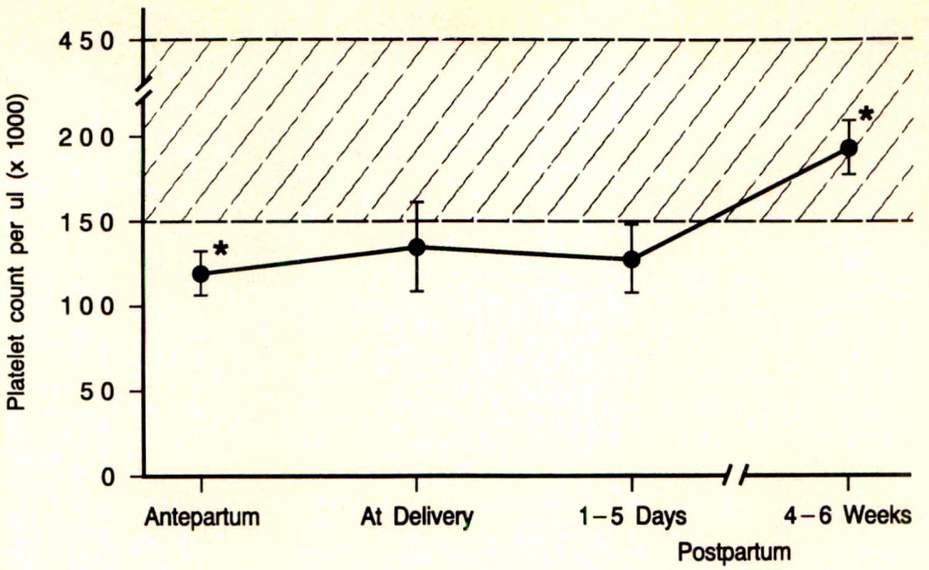


Fig. 1. Mean and SEM of platelet counts at various intervals from antepartum to postpartum periods. *Shaded areas* show normal range. *Asterisk* indicates highly significant, $p << 0.0001$.

Table II. Laboratory findings

	Platelet count × (10^3)				*Bleeding time (min)**		
Patient no.	*Lowest count AP*	*At delivery*	*1-5 Days PP*	*4-6 Weeks PP*	*AP*	*4-6 Weeks PP*	*Platelet antibodies*
1	77	144	152	239	4	—	Negative
2	98	218	200	274	10	7	1:2 (AP) Negative (PP)
3	140	186	160	191	5	—	1:16 (AP) 1:128 (1-5 days PP) 1:64 (4-6 wk PP)
4	115	106	124	166	7.5	—	Negative
5	54	76	90†	146	15	>15	1:64 (4-6 wk PP) 1:16 (3-4 mo PP)
6	121	134	127	194	6.5	—	Negative
7	95	104	78	166	11	>15	1:8 (4-6 wk PP) 1:4 (5 mo PP)
8	145	135	117	189	6.5	—	1:8 (AP) Negative (PP)
9	100	130	153	174	10.5	6	Negative
10	111	128	133	160	4	—	Negative
11	119	134	137	194	4	—	Negative
12	125	333	281	258	6	—	Negative
13	109	104	—	196	8	—	1:4 (4-6 wks PP)
14	113	80	101	167	6	—	Negative
15	70	97	78	159	10	6.5	Negative

AP, Antepartum; *PP*, post partum.
*Normal range of bleeding time is 2 to 8 minutes.
†Platelet count taken in the immediate postoperative period. Patient had received 10 units of platelet concentrate preoperatively.

patients (No. 3) had unbound platelet antibodies ante partum and post partum. Her titer was 1:16 at 25 weeks' gestation; it increased to 1:128 at 1 to 5 days post partum and decreased to 1:64 at 4 to 6 weeks post partum. Two other patients (Nos. 2 and 8) had unbound platelet antibodies detected only ante partum; their titers were 1:2 at 34 weeks' gestation and 1:8 at 37 weeks' gestation, respectively. Thrombocytopenia was not found in the infants born to these three mothers. The remaining three patients (Nos. 5, 7, and 13) had unbound platelet antibody detected during the postpartum period. Patient No. 5 had a titer of 1:64

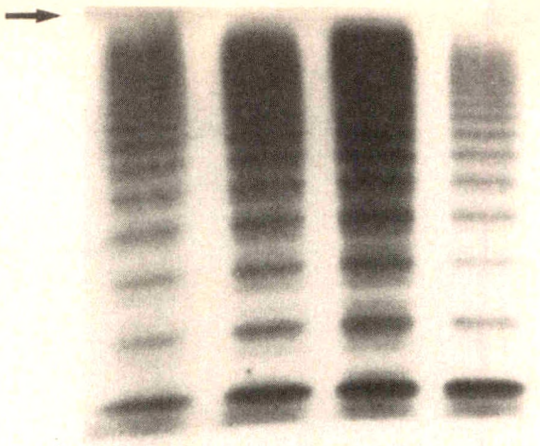


Fig. 2. Autoradiogram of sodium dodecylsulfate-agarose gel electrophoresis of plasma von Willebrand factor of patient No. 6. For comparison, multimeric distribution of pooled normal plasma *(PNP)* is shown on left. *Arrow* indicates top of running gel. Tests were run at different time intervals during antepartum and postpartum periods. Actual values of vWF:Ag and vWF:RiCof are shown at bottom.

Table III. Mean and SEM of von Willebrand factor antigen and ristocetin cofactor

	*Patients without vWD** (%)	*Patients with vWD†* (%)	*p Value of group A vs B*
Antepartum			
vWF:Ag	254 ± 33.2	156 ± 9.4	NS
vWF:RiCof	189 ± 27.9	112 ± 20.7	NS
Post partum (4 to 6 wk)			
vWF:Ag	143 ± 11.1	62 ± 8.1	<0.005
vWF:RiCof	108 ± 8.0	48 ± 2.0	<0.005
Post partum (5 to 6 mo)			
vWF:Ag	—	55 ± 12.7	—
vWF:RiCof	—	42 ± 7.0	—

vWD, von Willebrand disease.
Normal nonpregnant range: vWF:Ag = 50% to 150%; vWF:RiCof = 60% to 120%.
*n = 12.
†n = 3.

at 4 to 6 weeks post partum; it decreased to 1:16 at 3 to 4 months post partum. Patient No. 7 had a titer of 1:8 at 4 to 6 weeks post partum and 1:4 at 5 months post partum. Patient No. 13 had a low titer of 1:4 at 4 to 6 weeks post partum (Table II).

The antepartum values of vWF:Ag and vWF:RiCof in three patients (Nos. 5, 6, and 7) subsequently diagnosed with mild von Willebrand disease were lower but not significantly different from those of pregnant patients without this disorder. However, the postpartum values were significantly lower ($p < 0.005$). The diagnosis of von Willebrand disease was made 4 to 6 weeks post partum and was confirmed 5 to 6 months post partum (Table III). The analysis of von Willebrand factor multimers and associated laboratory data of patient No. 6 are shown in Fig. 2. There was no clinical reason to examine the patients without von Willebrand disease 5 to 6 months post partum.

One of 15 (6.6%) term infants was born with neonatal thrombocytopenia (78,000/μl; normal range, 100,000 to 280,000/μl).[10] The mother (patient No. 15) had thrombocytopenia (97,000/μl) and a prolonged bleeding time (10 minutes) with no other clinical abnormalities noted. The mean platelet count of the remaining 14 infants was 263,250 ± 27,796/μl.

The serum folate level, vitamin B_{12} level, prothrombin time, activated partial thromboplastin time, fibrinogen, fibrin split products, albumin level, and rheumatoid factor slide test results of all the patients studied were within normal limits. Similarly, urine toxicology screen was negative in all except patient No. 12 whose test was positive for cocaine and marijuana. Antinuclear antibody was positive for patients Nos. 1 and 8 (Table I).

Twelve of the 15 patients had a spontaneous vaginal delivery and three patients were delivered of infants by cesarean section. Patient No. 7 had decreasing serial platelet counts during the prenatal course. Her platelet counts were 118,000/μl at 25 weeks' gestation and 95,000/μl at 37 weeks. Cordocentesis was recommended to rule out the possibility of an immune-mediated thrombocytopenia. However, a low transverse cesarean section had to be performed when fetal bradycardia in association with hemorrhage at the umbilical cord's puncture site was noted. Consequently, no cordocentesis was recommended for subsequent patients unless immune thrombocytopenic purpura was definitely suspected. Patient No. 9 had a low transverse cervical cesarean section because of failure to progress. Patient No. 5 received 10 units of platelet concentrates 30 minutes before a repeat cesarean section to correct a prolonged bleeding time. None of these 15 patients, including those with a subsequent diagnosis of von Willebrand disease, had peripartum hemostatic complications.

Comment

Platelet counts in pregnant women remain within the established range even in the presence of hemodilution, placental trapping, or platelet hyperdestruction.[5, 11] However, recent publications have reported low platelet counts during uncomplicated pregnancy with no overt consequence to the mother and fetus.[1, 2, 4] The cause of the mild transient thrombocytopenia is still unknown.

Von Willebrand factor plays a role in platelet adherence to subendothelium and aggregation in response to ristocetin and has been implicated in the pathogenesis of thrombocytopenia in patients with clinical disorders such as thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, and preeclampsia-eclampsia. A similar mechanism could explain the mild thrombocytopenia observed during some pregnancies. von Willebrand factor is synthesized in endothelial cells and found in megakaryocytes. It is stored in Weibel-Palade bodies of endothelial cells and α-granules of platelets. Under certain physiologic conditions, such as stress or pregnancy, von Willebrand factor is released into circulation or deposited in the subendothelium as multimers of variable molecular weight. The biologic activities of von Willebrand factor include platelet-to-platelet interaction and platelet adhesion to the vessel wall. It also functions as a carrier protein for Factor VIII coagulant in plasma. Quantitative and qualitative abnormalities of von Willebrand factor cause a bleeding disorder known as von Willebrand disease. Its main symptoms are easy bruising, severe menstrual bleeding, and prolonged bleeding after injury or surgery. The diagnosis usually is established by a family history of a bleeding diathesis, a prolonged bleeding time, and low levels of vWF:Ag and vWF:RiCof. The classification of von Willebrand disease into subtypes is made on the basis of the multimeric characteristics of von Willebrand factor. In type I, the most common form, vWF:Ag and vWF:RiCof are decreased in parallel and all multimers are present to a lesser degree. In type II, the high molecular weight forms are missing.[12]

No patient was thought to have von Willebrand disease on the basis of her history; however, because bleeding time and von Willebrand factor activity were part of the evaluation, these tests helped us diagnose the disorder in three patients. The thrombocytopenia improved in the postpartum period in patients both with and without von Willebrand disease. A follow-up of the mean platelet volume did not correlate with the shift in platelet counts, making hemodilution a more likely cause than an increase in platelet turnover. Because thrombocytopenia was diagnosed during pregnancy, we did not subject the patients to radiolabeled platelet studies to determine platelet survival. The bleeding time of the three patients with von Willebrand disease was persistently prolonged throughout the antepartum and postpartum period in two patients and normal in one patient. However, the latter was started on birth control pills 2 weeks post partum and hence may have had her expected prolonged bleeding time masked during the postpartum period. The platelet counts of two of the three patients with von Willebrand disease returned to normal during the postpartum period. Conti et al.[13] also reported mild thrombocytopenia in one patient with type I von Willebrand disease during pregnancy. When type I von Willebrand disease is present during pregnancy, desmopressin (1-Desamino-8-D-arginine-vasopressin, DDAVP) 0.4 μg/kg may be given 30 minutes before delivery to prevent or decrease potential hemostatic complications.[14]

To extend diagnostic possibilities, we measured unbound platelet antibodies. The level of maternal

unbound platelet antibodies in patients with immune thrombocytopenic purpura has been correlated with both the presence and the extent of neonatal thrombocytopenia[15]; however, the level's predictive value is confounded by the presence of measurable levels of maternal and umbilical cord unbound platelet antibodies because of nonspecific activity found in a normal pregnancy.[16] This was evident in our study; three mothers with unbound platelet anibodies detected during prenatal period were delivered of three infants with normal platelet counts. The detection of unbound platelet antibodies in one of the patients post partum may have resulted from preoperative platelet transfusions. The other two patients whose unbound platelet antibody was detected only post partum may have had an antigen-antibody reaction resulting from fetomaternal transfer of heterogeneous platelet antigen that might have occurred during pregnancy.

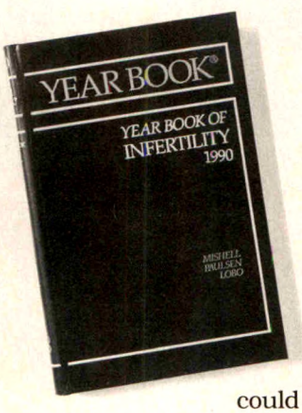
We found the incidence of thrombocytopenia to be 0.4% at the time of the first visit. This apparent low incidence compared with the other recent articles may be a result of the design of the study in which thrombocytopenia that developed in patients later in the course of pregnancy could be missed.

In conclusion, our study suggests that incidental, mild thrombocytopenia during pregnancy is often benign and transient. Extensive testing, such as those mentioned in our Material and methods section, is not indicated in such cases unless there is an associated prolonged bleeding time or persistent thrombocytopenia 4 to 6 weeks post partum. von Willebrand disease should then be considered. Three of the 15 patients had mild type I von Willebrand disease; however, because vWF:Ag and vWF:RiCof levels were elevated during pregnancy, the diagnosis could be made only in the postpartum period. Repeat platelet count at 6 to 8 weeks interval to observe its trend may be performed as clinically indicated. From the results of our study, neonatal thrombocytopenia seems unlikely to occur in the presence of mild transient maternal thrombocytopenia. In our series only one term infant showed mild thrombocytopenia. This indicates that vaginal delivery is safe and that abdominal delivery should be performed only in the presence of obstetric indications.

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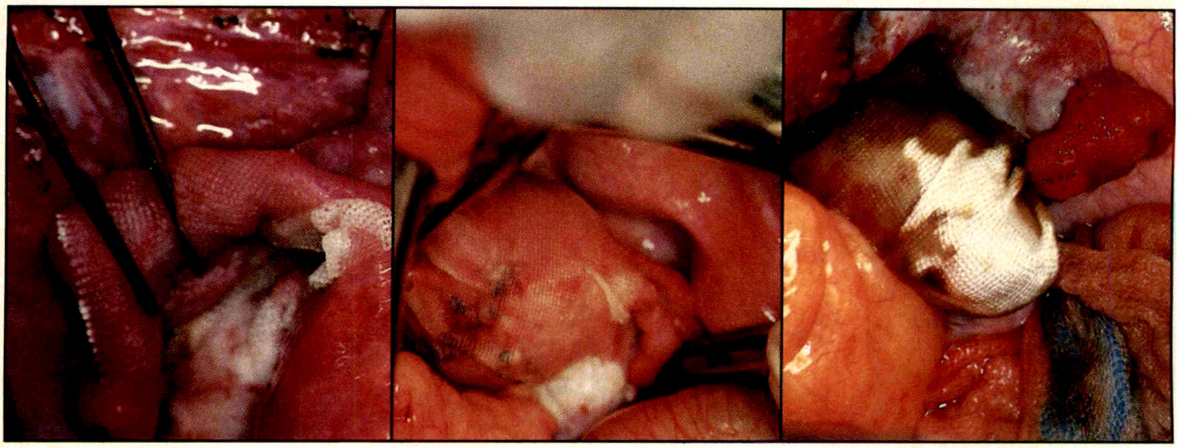
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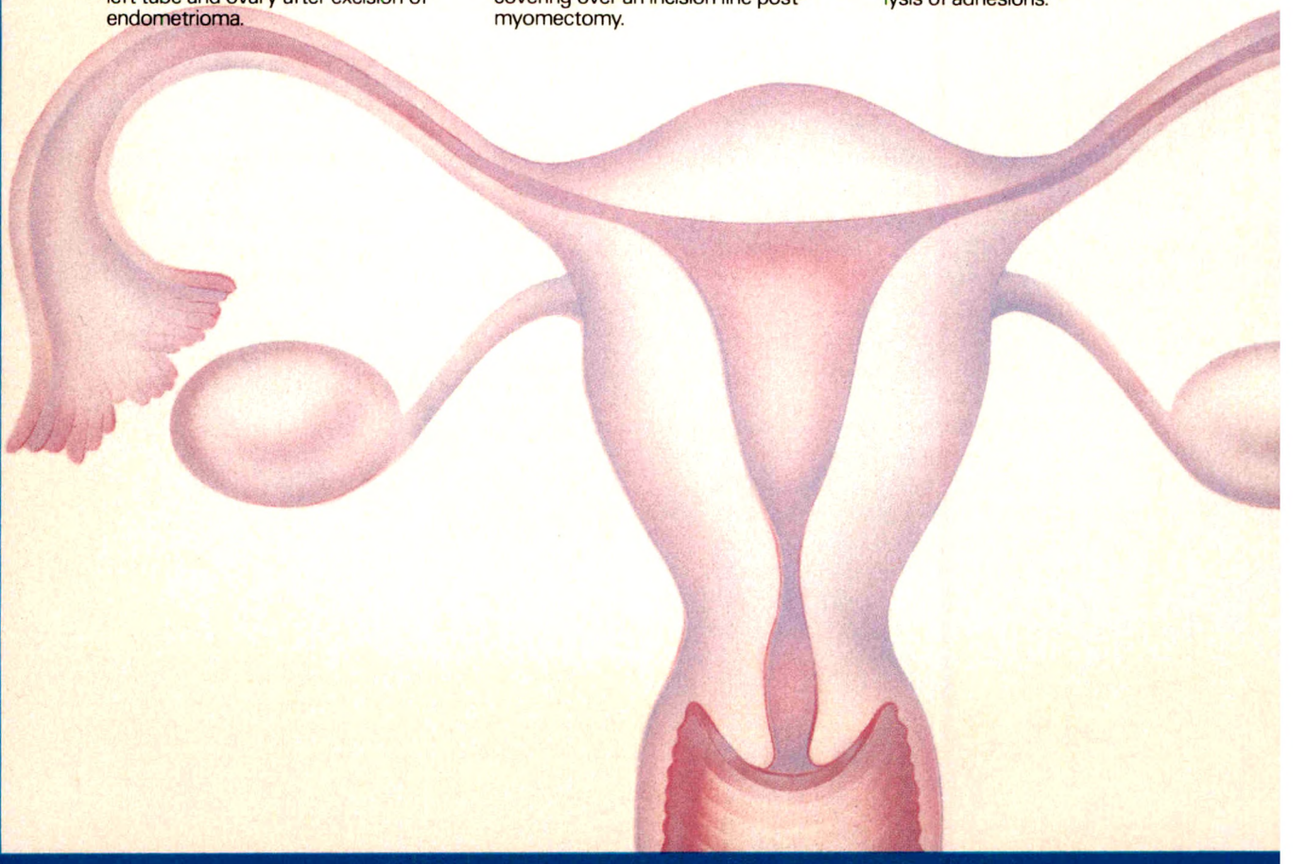
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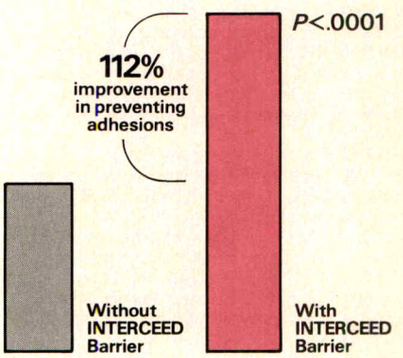
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The clinical implications of subchorionic placental lucencies

Vern L. Katz, MD, George F. Blanchard, Jr., BA, RDMS, William J. Watson, MD, Richard C. Miller, MD, Nancy C. Chescheir, MD, and John M. Thorp, Jr., MD

Chapel Hill, North Carolina

Forty-six patients were identified at a perinatal diagnostic referral center as having subchorionic placental lucencies. These lucencies varied from simple to multicystic and covered up to 50% of the subchorionic placental surface. Clinical follow-up of 40 patients demonstrated no increase in the incidence of adverse pregnancy outcome. We conclude that these pregnancies are not at higher risk when the subchorionic placental lucencies are not associated with other intraplacental or fetal anomalies. (Am J Obstet Gynecol 1991;164:99-100.)

Key words: Placenta, ultrasonography, subchorionic lucencies

Subchorionic placental lucencies are a common cause of referral for detailed ultrasonographic examination and/or perinatal consultation. Spirt et al.,[1] in 1978, evaluated 10 patients with subchorionic placental lucencies of various sizes and obtained histologic diagnoses. These lucencies corresponded to areas of subchorionic fibrin deposition, a normal finding in 10% to 15% of pregnancies.[2] This investigation sought to evaluate the clinical implications of large subchorionic placental lucencies detected on ultrasonography to assess the importance and need for consultation.

Methods

Over a 6-month period, 1856 patients were referred for detailed ultrasonographic evaluation. Patients with greater than 2 × 2 cm subchorionic placental lucencies were identified prospectively and clinical outcomes were obtained. Cases were managed as clinically appropriate for respective referral indications.

Results

In this group of 56 referral patients, 18 were identified as having subchorionic lucencies. Of these, 14 were referred for evaluation of fetal anatomy, 10 because of increased maternal age, 8 because of low maternal serum α-fetoprotein level, 6 for examination of placental anomalies, 4 because of elevated serum α-fetoprotein level, and 4 because of maternal diabetes. The mean gestational age at examination was 19.3 weeks (range 13 to 34 weeks).

Representative illustrations of the placental lucencies are shown in Fig. 1. Lucencies varied from simple to multicystic. None were immediately below the umbilical cord insertion, and only one was associated with an intraplacental lucency. The lucencies covered from 10% to 50% of the placental surface by estimation and were from 2 to 6 cm in longest dimension.

Follow-up was obtained on 40 of 46 pregnancies. There were two perinatal deaths, one in association with neonatal complications of immune hydrops in an Rh-sensitized patient and one in conjunction with a premature birth in a twin pregnancy. Neither death appeared to be related to placental abnormality. The second twin is alive and thriving. One patient had premature labor at 32 weeks with delivery at term. One patient had meconium-stained amniotic fluid. There were seven cesarean births (17%), similar to occurrence in the general obstetric population. No patient reported bleeding in the third trimester. One patient had pregnancy-induced hypertension at term. All 14 amniocenteses produced normal karyotypes and normal α-fetoprotein levels. There were two congenital anomalies observed in the 46 fetuses, a mild unilateral hydronephrosis found at ultrasonography and a dislocated hip found at birth.

Comment

Subchorionic placental lucencies do not appear to be associated with adverse pregnancy outcomes. The clinical course of this group of patients is similar to what would be expected in the general population. The incidence of elevated maternal serum α-fetoprotein in patients with lucencies was similar to that of our referral

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of North Carolina School of Medicine.

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Reprint requests: Vern L. Katz, MD, Maternal-Fetal Medicine, CB# 7570, 214 MacNider Bldg., UNC School of Medicine, Chapel Hill, NC 27599-7570.

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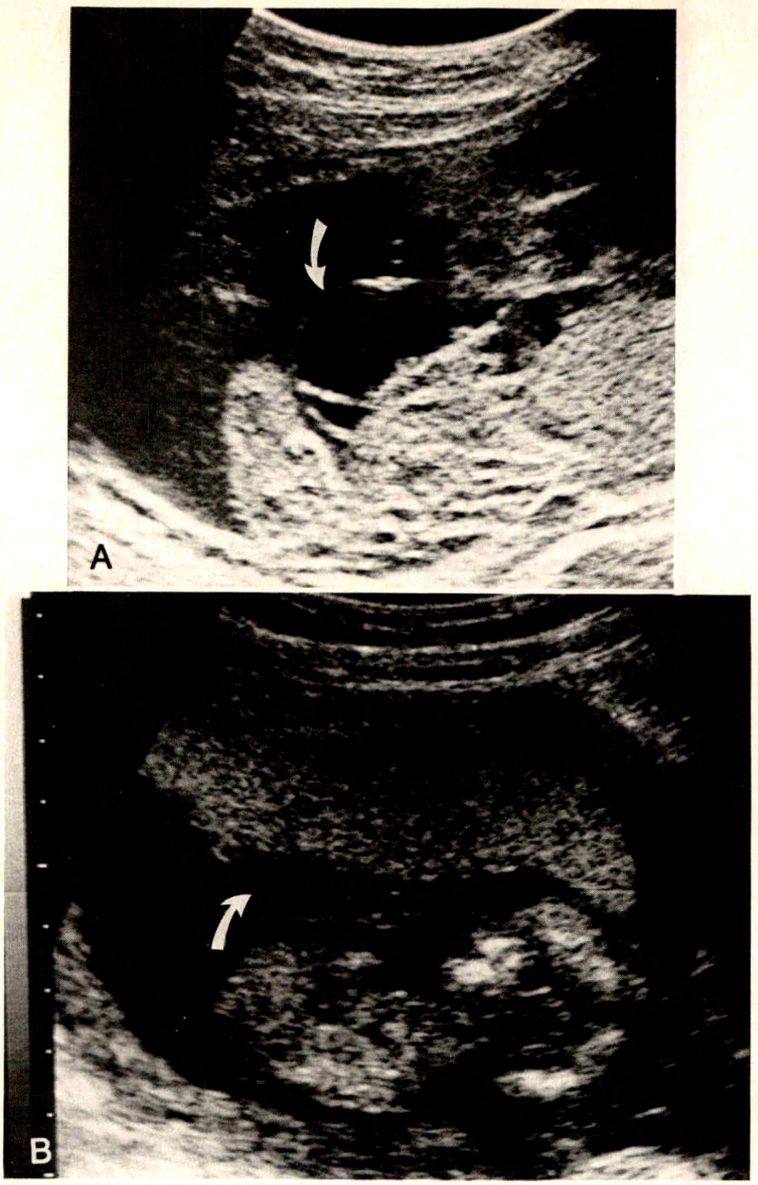


Fig. 1. A and **B**, Ultrasonograms of subchorionic placental lucencies. *Arrows* point to subchorionic lucencies.

population. Thus this ultrasonographic finding does not seem to be associated with elevated maternal serum α-fetoprotein levels. Detailed ultrasonographic and/or perinatal consultation does not appear to be necessary for subchorionic placental lucencies provided the following criteria are met: The lucencies do not appear to have flow within them as seen with chorioangiomas, and there are no associated fetal anomalies or other placental abnormalities identified at the time of ultrasonographic evaluation.

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The transverse cerebellar diameter in the second trimester is unaffected by Down syndrome

Lyndon M. Hill, MD,[a] Dawn Rivello, RDMS,[a] Carol Peterson, RDMS,[a] and Sandra Marchese, MS[b]

Pittsburgh, Pennsylvania

Cerebellar hypoplasia is a consistent finding in necropsy studies of children and adults affected by Down syndrome. The transverse cerebellar diameter was measured in 23 second-trimester fetuses with Down syndrome. All the measurements were within the normal range for gestational age. (AM J OBSTET GYNECOL 1991;164:101-3.)

Key words: Transverse cerebellar diameter, Down syndrome

Advanced maternal age is by far the most frequently given indication for genetic amniocentesis. However, <20% of children with Down syndrome are born to women over 35 years of age.[1] Consequently, the use of maternal age alone is an extremely ineffective screening tool. Merkatz and coworkers[2] were the first to discover an association between low maternal serum α-fetoprotein levels and chromosomal aneuploidies. Corroboration of their findings by others,[3, 4] resulted in the current practice of adjusting a patient's risk of having a child with Down syndrome according to maternal age and maternal serum α-fetoprotein level. Unfortunately, this technique has a low sensitivity (13% to 32%) and a high false-positive rate (4.1% to 9%).[3, 5]

As the resolution of ultrasonographic equipment continues to improve, increasingly finer anatomic detail can be appreciated. The growth patterns of different fetal parts have been documented. Hence, deviations in fetal growth, both in the time of onset and in degree, can be documented through serial ultrasonography. With this information, chromosomal or physical abnormalities that affect fetal growth can be detected.

The central nervous system of children and adults with Down syndrome has been extensively evaluated. The abnormalities that have been identified are varied and inconstant. However, Davidoff[6] in an autopsy study of 10 cases of Down syndrome consistently found a small cerebellum. Therefore we sought to evaluate the transcerebellar diameter in fetuses with Down syndrome to determine whether there is a reduction in cerebellar size by the second trimester of pregnancy.

From the Departments of Ultrasound[a] and Human Genetics,[b] Magee-Womens Hospital, University of Pittsburgh.
Received for publication June 28, 1990; revised August 6, 1990; accepted August 15, 1990.
Reprint requests: Lyndon M. Hill, MD, Magee-Womens Hospital, Forbes Ave. and Halket St., Pittsburgh, PA 15213.
6/1/24744

Material and methods

We retrospectively ascertained all women with a gestational age between 15 and 20 weeks who had undergone a level II ultrasonographic examination and genetic amniocentesis at Magee-Womens Hospital between February 1987 and November 1989.

The following dating criteria were required to enter the study: regular menses, no oral contraceptives for 3 months before conception, an early pregnancy test, and a first examination at or before 10 weeks' gestation. Exclusion criteria included insufficient dating parameters, an intrauterine fetal death before the ultrasonographic examination, or failure to image and measure the transverse cerebellar diameter.

Of the 28 fetuses with a karyotypic diagnosis of Down syndrome, 23 met the entrance criteria. All the pregnancies were terminated after a diagnosis of Down syndrome was made.

A thorough ultrasonographic examination was performed with a linear-array real-time system with a 3.5 or a 5.0 MHz transducer (General Electric RT 3000, RT 3200; General Electric Corporation, Milwaukee, Wis.). A composite assessment of gestational age was obtained from standard biometric measurements of the biparietal diameter, femur length, head circumference, and abdominal circumference. The amount of amniotic fluid and the placental site were documented.

We have consistently measured the transverse cerebellar diameter as part of our routine anatomic survey since 1986. A slight posterior angulation from the level of the biparietal diameter permits visualization of the cerebellum (Fig. 1).[7] Hence, whereas we retrospectively accumulated our study group, the transverse cerebellar diameter was measured prospectively. The normal cerebellar diameter for our population as a function of gestational age was derived prospectively from 296 normal pregnant patients with the noted dating criteria

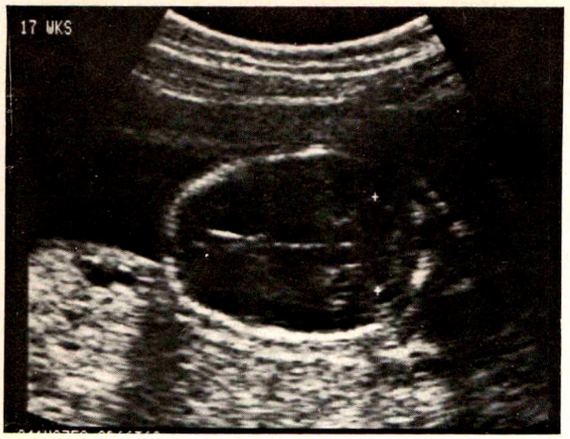


Fig. 1. Graticules outline transverse cerebellar diameter at 17 weeks' gestation.

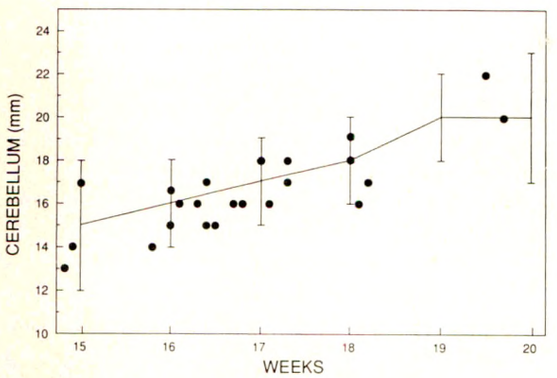


Fig. 2. Normal (± 2 SD) transverse cerebellar diameter from 15 to 20 weeks' gestation. *Filled circles* represent fetuses with Down syndrome.

between 15 to 20 weeks' gestation. Each fetus provided a single data point for the reference curve.

Results

The mean cerebellar diameter (± 2 SD) between 15 and 20 weeks' gestation is presented in Fig. 2. The 23 cases of Down syndrome are also plotted in Fig. 2. It can be seen that the transverse cerebellar diameter is unaffected by Down syndrome in the second trimester.

Comment

Advanced maternal age and decreased maternal serum α-fetoprotein levels are accepted risk factors for Down syndrome. In 1981, approximately 20% of Down syndrome cases were associated with a maternal age >34 years.[1] As women continue to delay child bearing into their midthirties to late thirties, the percentage of Down syndrome newborns will increase.

A second-trimester ultrasonographic examination is not a routine part of antenatal care in the United States. However, it has been estimated that in an average practice, approximately 60% of the patients will require an ultrasonographic examination on the basis of history or physical examination at some point in their pregnancy.[8] The use of ultrasonography in obstetrics will only continue to increase in the future. Hence, the determination of any reliable ultrasonographic signs for Down syndrome might have widespread application.

The finding of a normal transverse cerebellar diameter in fetuses with Down syndrome during the second trimester appears to be at odds with the significantly smaller cerebellar weight found in children and adults with Down syndrome. This paradox can be explained by the time period during which cerebellar development is affected. The two major periods of cellular multiplication in the human brain are from 15 to 20 weeks and from 25 weeks until 2 to 4 years of age. The first phase of multiplication represents proliferation of neuroblasts, whereas the second phase is primarily concerned with glial division and neuroblast maturation.[12] The neuroblasts differentiate into interstitial neurons, Golgi cells and Purkinje cells. Although the Purkinje cells obtain their full number at birth, they are immature and undeveloped. The subsequent differentiation of the Purkinje cells during childhood plays a major role in the development of the cerebellum. Cerebellar hypoplasia may result if this process is inhibited.[13] Degenerative processes after birth also appear to play a role in the reduction of brain size that has been consistently noted with Down syndrome.[10]

In conclusion, cerebellar hypoplasia in neonates and adults with Down syndrome appears to occur after the second trimester. Consequently, measurement of the transverse cerebellar diameter before 20 weeks' gestation cannot be used as an ultrasonographic sign for the detection of Down syndrome.

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Maternal postprandial glucose levels and infant birth weight: The Diabetes in Early Pregnancy Study

Lois Jovanovic-Peterson, MD,[a] Charles M. Peterson, MD,[a] George F. Reed, PhD,[b] Boyd E. Metzger, MD,[c] James L. Mills, MD, MS,[b] Robert H. Knopp, MD,[d] Jerome H. Aarons, MD,[c] and the National Institute of Child Health and Human Development—Diabetes in Early Pregnancy Study

Santa Barbara, California, Bethesda, Maryland, Chicago, Illinois, Seattle, Washington, and Pittsburgh, Pennsylvania

The cause of macrosomia in the infant of the diabetic woman is still not completely defined. The National Institute of Child Health and Human Development—Diabetes in Early Pregnancy Study, which recruited insulin-dependent diabetic and control women before conception, provided an opportunity to address the relationship between maternal glycemia and percentile birth weight. Data were analyzed from 323 diabetic and 361 control women. Fasting and nonfasting venous plasma glucose were measured on alternate weeks in the first trimester and monthly thereafter. Glycosylated hemoglobin was measured weekly in the first trimester and monthly thereafter. More infants of the diabetic women were at or above the 90th percentile for birth weight than infants of control women (28.5% versus 13.1%, $p < 0.001$). Although first-trimester nonfasting glucose and glycosylated hemoglobin levels were positively correlated with infant birth weight ($p < 0.001$ and $p = 0.008$), when the analyses were adjusted for the variables of the subsequent trimesters the values became insignificant, whereas the third-trimester nonfasting glucose levels adjusted for values in prior trimesters emerged as the stronger predictor of percentile birth weight ($p = 0.001$). After adjusting for maternal hypertension, smoking, and ponderal index, the above relationships remained. In conclusion, monitoring of nonfasting glucose levels rather than the fasting levels, which are more commonly monitored in clinical practice, are necessary to prevent macrosomia. (AM J OBSTET GYNECOL 1991;164:103-11.)

Key words: Type I diabetes mellitus, macrosomia, postprandial glucose, glycosylated hemoglobin, pregnancy

Fetal macrosomia, defined as greater than the 90th percentile for weight, occurs in 25% to 42% of pregnancies in diabetic women as compared with 8% to 14% in control populations.[1] Macrosomic infants of diabetic women suffer increased morbidity and mortality from unexplained death in utero, birth trauma, hypertrophic cardiomyopathy, vascular thrombosis, neonatal hypoglycemia, hyperbilirubinemia, erythrocytosis, and respiratory distress. Therefore inquiries into the nature and causes of macrosomia are important to find means by which many of these complications may be avoided.

The Pedersen hypothesis[2] states that maternal hyperglycemia stimulates the fetal pancreas to secrete excess insulin, which produces *inter alia* adiposity and macrosomia. Although there is increasing evidence that

From the Sansum Medical Research Foundation,[a] the National Institute of Child Health and Human Development,[b] Northwestern University,[c] the University of Washington,[d] and the University of Pittsburgh.[e]
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Reprint requests: Lois Jovanovic-Peterson, MD, the Sansum Medical Research Foundation, 2219 Bath St., Santa Barbara, CA 93105.
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Table I. Maternal variables

Variables	Diabetic		Control		p Value
	Mean	SD	Mean	SD	
Height (cm)	163.1	7.2	164.1	7.3	0.103
Weight (kg)	63.0	9.7	62.7	12.3	0.726
Age (yr)	27.9	4.1	29.6	3.7	<0.0001

Table II. Infant percentile birth weight

Percentile	Diabetic		Control	
	No.	%	No.	%
<3	8	2.5	6	1.7
3-9	17	5.3	20	5.5
10-24	24	7.4	51	14.1
25-49	41	12.7	82	22.7
50-74	92	28.5	86	23.8
75-89	49	15.2	69	19.1
90-96	54	16.7	28	7.8
97	38	11.8	19	5.3
TOTAL	323	—	361	—

maternal glucose levels influence fetal growth, there is still controversy as to its importance in causing macrosomia.[3] There is now evidence in support of modifying the Pedersen hypothesis to include additional fuels as causal agents for macrosomia.[4]

On the one hand, there are reports that despite "normoglycemia" macrosomia remains more common in pregnancies complicated by diabetes than in the normal population.[5-7] On the other hand, a number of groups have reported that meticulous efforts to achieve tight control of diabetes can normalize birth weight in infants of diabetic mothers.[8-11] Glycemic control in insulin-dependent diabetic women is particularly difficult to assess. In most prior reports, data are available about glycemic levels in the last half of pregnancy, but lacking in early pregnancy.[12-15] Glycosylated hemoglobin levels provide some information but reflect glucose levels for only the previous 8 to 10 weeks.[16] Furthermore, most studies reflect clinical practice inasmuch as fasting and preprandial glucose values are more often measured than postprandial values.

The National Institute of Child Health and Human Development—Diabetes in Early Pregnancy Study was a multicenter study in which control and diabetic women were recruited before or within 21 days after conception and in which glycemic control was documented by fasting, nonfasting, and glycosylated hemoglobin levels throughout pregnancy.[17] Thus the Diabetes in Early Pregnancy Study provided a unique opportunity to study the relationship between maternal glycemia and infant birth weight.

Methods

The Diabetes in Early Pregnancy Study included Cornell University, Harvard University, Northwestern University, the University of Pittsburgh, and the University of Washington. The National Institute of Child Health and Human Development was the data and coordinating center. The study design has been described in detail elsewhere.[17, 18] When the diagnosis of pregnancy was made, diabetic subjects were hospitalized for 2 to 5 days to monitor metabolic control. Other than reinforcing the techniques of self-monitoring of blood glucose levels and charting the results four times a day (fasting and 1 hour after each meal), a standard approach to the management of diabetes was not imposed; nor were fixed goals for glycemic control mandated. After discharge, diabetic subjects were monitored at weekly clinic visits from weeks 5 through 12 after the last menstrual period and monthly thereafter. At each clinic visit analyses of glycosylated hemoglobin and plasma glucose were performed. Nonfasting glucose was defined as 1 hour after the meal, which represents the highest blood glucose after the meal.[19] Control subjects were screened for gestational diabetes at 26 weeks' gestation according to the criteria establish by the Second International Gestational Diabetes Workshop.[20] Three gestational diabetic women were identified and included in the analysis.

The thiobarbituric acid method was used for glycosylated hemoglobin analysis.[21] This method is not affected by fetal hemoglobin and made it possible to perform all the tests on frozen, washed red blood cells in a single laboratory. Laboratory plasma glucose measurements were performed in each center by an enzymatic method. Although it is acknowledged that routine clinical laboratory analysis of glucose is not always accurate, each center used a certified laboratory and provided unknown samples as spot checks.

The infants' birth weight, gestational age at the time of delivery as determined by four parameters (last menstrual period, basal body temperature charting, ultrasonography at 8 and 12 weeks, and Dubowitz examination of the newborn),[17] gender, and race were used to calculate the infants' percentile birth weight. National natality data were used to construct standards

Table III. Mean fasting, nonfasting and glycohemoglobin values for each trimester for each percentile grouping

Percentile grouping	First trimester			Second trimester			Third trimester		
	Fasting	Nonfasting	HbA_{Ic}	Fasting	Nonfasting glucose	HbA_{Ic}	Fasting	Nonfasting glucose	HbA_{Ic}
>97	108	120	69.6	137	165	64.5	126	145	63.0
90-96	96	125	69.0	124	133	64.0	117	137	62.3
75-89	94	102	68.5	110	127	63.9	119	122	62.0
50-74	101	115	68.0	129	134	63.1	125	120	61.2
25-49	98	101	67.0	114	108	63.0	109	105	60.9
10-24	87	90	67.2	111	102	62.0	97	107	60.4
3-9	95	90	67.1	108	118	62.0	113	83	60.0
<3	109	83	67.0	126	118	61.8	104	79	59.9

Hb, Hemoglobin.

Table IV. The relationship between metabolic parameters and birth weight in diabetic women when each trimester's variables are analyzed independently

	No.	B* + SE	p Value
Fasting glucose			
First trimester	289	0.002 ± 0.002	0.248
Second trimester	259	0.006 ± 0.002	0.024
Third trimester	238	0.005 ± 0.003	0.068
Nonfasting glucose			
First trimester	316	0.006 ± 0.002	<0.001
Second trimester	137	0.006 ± 0.002	0.02
Third trimester	132	0.013 ± 0.004	<0.001
Glycosylated hemoglobin			
First trimester	318	0.025 ± 0.009	0.008
Second trimester	240	0.032 ± 0.012	0.009
Third trimester	219	0.047 ± 0.013	<0.001

*Where B equals the slope of the association and *p* values are the result of testing whether there is no effect of the factor on birth weight category in a logistic regression equation.

Table V. Relationship between metabolic parameters and birth weight in diabetic women when each trimester's variables are adjusted for variables in subsequent trimesters

	No.	B* + SE	p Value
Fasting glucose†	221	<0.001 ± 0.003	0.937
Nonfasting glucose†	92	0.003 ± 0.005	0.581
Glycosylated hemoglobin†	199	0.019 ± 0.020	0.343

*Where B equals the slope of the association and *p* valves are the result of testing whether there is no effect of the factor on birth weight category in a logistic regression equation.

†First trimester controlling for second and third trimesters.

Table VI. The relationship between metabolic parameters and birth weight in diabetic women when the second and third trimesters are adjusted for the variables of the prior trimester(s)

	No.	B* + SE	p Value
Fasting glucose			
Second trimester†	247	0.006 ± 0.003	0.035
Third trimester‡	221	0.001 ± 0.003	0.703
Nonfasting glucose			
Second trimester†	136	0.003 ± 0.003	0.285
Third trimester‡	92	0.014 ± 0.005	0.009
Glycosylated hemoglobin			
Second trimester†	235	0.003 ± 0.19	0.883
Third trimester‡	199	0.057 ± 0.24	0.018

*Where B equals the slope of the association and *p* values are the result of testing whether there is no effect of the factor on birth weight category in a logistic regression equation.

†Controlling for first trimester.

‡Controlling for second and first trimesters.

for birth weight percentile groups. Race, gender, and gestational age–specific standards were generated from all births in the United States from 1983.[22] Infants were classified into the following percentile birth weight groupings: <3, 3 to 9, 10 to 24, 25 to 49, 50 to 74, 75 to 89, 90 to 96, and ≥97th percentile. Each Diabetes in Early Pregnancy Study subject was then placed in the appropriate percentile group for the analysis. The diabetic and control subjects were compared for demographic, medical, and social factors known to influence the risk of either a small-for-gestational-age or a large-for-gestational-age infant[1] (Table I).

Statistical analyses. Logistic regression for categoric outcomes[23] was used to examine effects of glycemic control (calculated from the mean levels of fasting glucose, nonfasting glucose, and glycosylated hemoglobin for each woman for that trimester). Initially, the effect of glycemic control in each trimester of pregnancy was examined separately. Then, the effect of control in each trimester was examined adjusting for control in prior or subsequent trimesters. A total of 221 diabetic women had fasting glucose determinations for all three trimes-

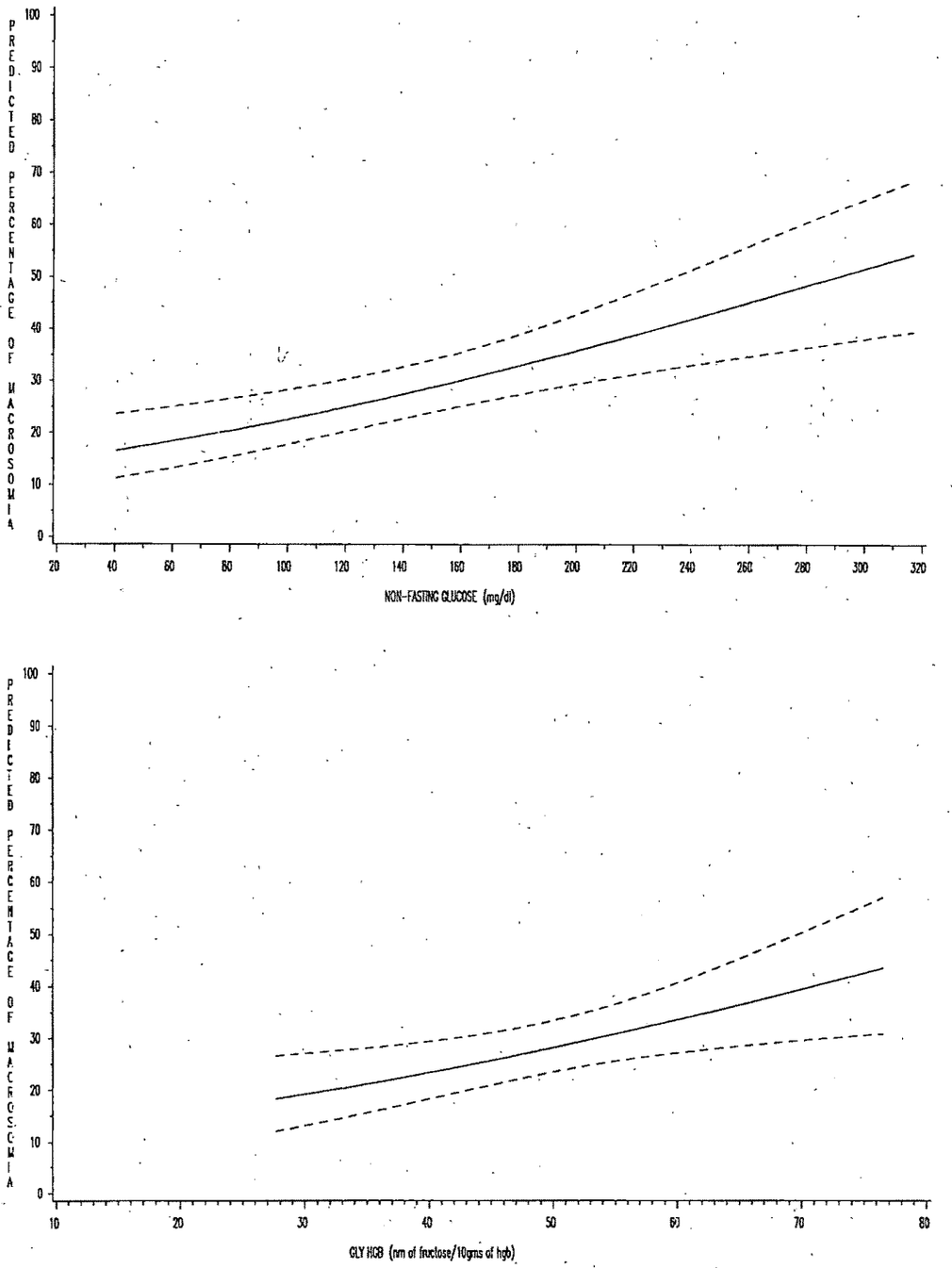


Fig. 1. *Top panel,* Risk of macrosomia (and 95% confidence interval) on basis of first-trimester nonfasting glucose levels in diabetic subjects. *Bottom panel,* Risk of macrosomia (and 95% confidence interval) on basis of first-trimester glycosylated hemoglobin levels in diabetic subjects (normal = mean ± SD = 38 ± 5 nmol fructose/10 gm hemoglobin).

ters; 92 had nonfasting glucose determinations for all three trimesters; and 199 had glycosylated hemoglobin determinations for all three trimesters. To verify that those women who had elevated glucose levels in the first trimester were the same women who had elevated levels in the third trimester, the first and third trimester glucose variables were analyzed by logistic regression.

The regression coefficients corresponding to the

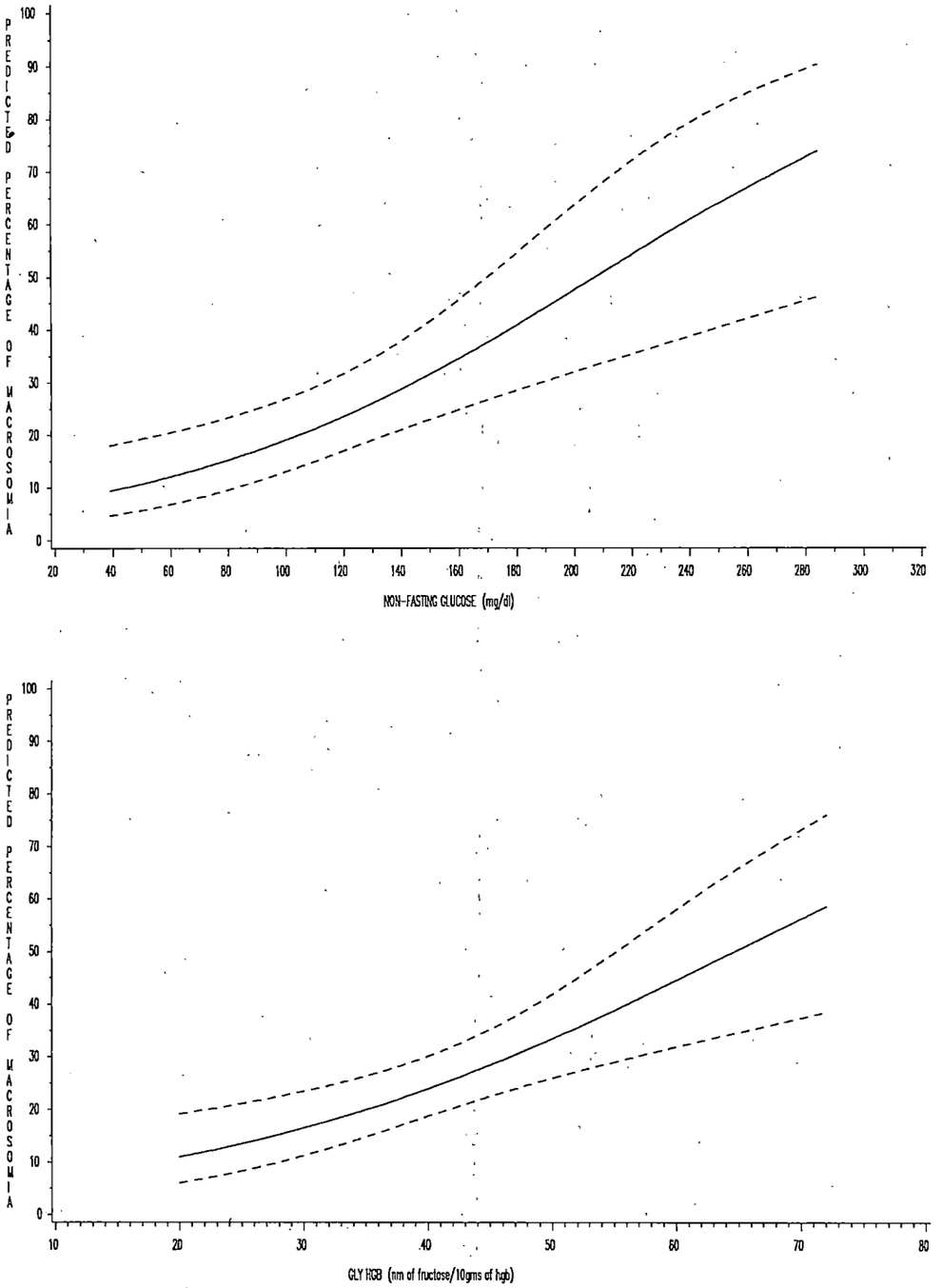


Fig. 2. *Top panel,* Risk of macrosomia (and 95% confidence interval) on basis of third-trimester nonfasting glucose levels in diabetic subjects. *Bottom panel,* Risk of macrosomia (and 95% confidence interval) on basis of third-trimester glycosylated hemoglobin levels in diabetic subjects (normal = mean ± SD = 38 ± 5 nmol fructose/10 gm hemoglobin).

trimester effects are expressed as logarithm odds ratios. A positive value indicates that as the mean glucose or glycosylated hemoglobin increases, then the odds of a birth occurring in a given or higher weight class increases relative to the odds of its occurring in a lower class; a negative value indicates that the odds for being in a higher class decreases in comparison to the odds for being in a lower weight class. Thus significant and

Table VII. Hospital and self-monitored nonfasting blood glucose levels in each trimester*

Glucose (mg/dl)	No.			Mean			Std Dev		
	1	2	3	1	2	3	1	2	3
Hospital	2421	1231	529	142	126	113	75	59	45
Home	788	386	148	133	113	99	71	53	41

Std Dev, Standard deviation. First trimester: $r = 0.81, p < 0.001$; second trimester: $r = 0.77, p < 0.001$; third trimester: $r = 0.82$, $p < 0.001$.

*Spearman correlation coefficients (glucose hospital levels compared with glucose home levels).

positive coefficients mean that the proportion of births in higher weight classes increases with the independent (glycemic control) variable.

These analyses were then adjusted for five known risk factors for producing either a small-for-gestational-age infant or a large-for-gestational-age infant. They were entered singly because of the relatively small numbers of observations. The five risk factors were as follows: (1) maternal hypertension (blood pressure >140/90 mm Hg) documented at any time during the pregnancy, (2) renal dysfunction as defined as a creatinine clearance <70 ml/min (White class F or RF), (3) maternal smoking during pregnancy, (4) maternal ponderal index (as defined as weight/height × 100) at the first clinic visit after pregnancy was diagnosed (week 5), and (5) maternal weight gain >20 kg by 32 weeks' gestation. Other statistical comparisons were made by the Student *t* test, Spearman correlation coefficients, and Wilcoxon rank sum test. The self-monitored blood glucose results were analyzed in the same fashion as the analysis for the laboratory-determined glucose levels.

Results

The entire data set comprised 323 diabetic and 361 control patients who were delivered of live infants. Table I shows the comparison between diabetic and control groups for maternal height, weight, and age. The diabetic and control mothers were not different for height and weight, but the control mothers were older. Table II shows the distribution of infant percentile birth weight for diabetic and control subjects. Infants of diabetic mothers were in the higher percentiles for body weight. The diabetic group had 92 (28.5%) infants and the control group had 47 (13.1%) infants at or above the 90th percentile ($p < 0.001$).

Table III shows the relationship between the laboratory mean levels of maternal fasting glucose, nonfasting glucose, glycosylated hemoglobin, and the infant percentile birth weight in the diabetic group. The mean of each trimester's variables were analyzed independently. When the fasting plasma glucose level was related to the percentile birth weight, only the second-trimester fasting glucose level showed a significantly positive relationship ($p = 0.024$) (Table IV). The relationship of nonfasting glucose to infant birth weight was significantly positive in each of the three trimesters. The first- and third-trimester nonfasting plasma glucose levels achieved the highest positive significant correlation with percentile birth weight ($p < 0.001$). The relationship between birth weight and level of glycosylated hemoglobin was significant for all trimesters, but the highest degree of significance was seen in the third trimester ($p < 0.001$). Thus when mean values of each trimester were analyzed separately, the first- and third-trimester nonfasting plasma glucose level and the third-trimester glycosylated hemoglobin level emerged with the strongest correlation to subsequent infant birth weight.

Figs. 1 and 2 show the logistic regression of the predicted risk of macrosomia. The top panels use the mean of the diabetic subjects' mean nonfasting glucose levels and the bottom panels use the mean of the diabetic subjects' mean values of glycosylated hemoglobin. As can be seen in Fig. 1, a first-trimester nonfasting blood glucose level >120 mg/dl increases the risk of macrosomia by 24%. Likewise, if the first-trimester glycosylated hemoglobin level is >44 nmol of fructose per 10 gm of hemoglobin (the 95th percentile for the normal control population), the risk of macrosomia is 25%. Fig. 2 shows the mean third-trimester nonfasting and glycohemoglobin values. A mean third-trimester nonfasting glucose level >120 mg/dl increases the risk 23%; similarly a glycohemoglobin level >44 nmol fructose/10 gm hemoglobin increases the risk to 32%.

When the first-trimester mean levels of fasting glucose, nonfasting glucose, and glycosylated hemoglobin were controlled for the values in the second and third trimesters, the effect of the subsequent trimesters eliminated the significant relationship of the first-trimester variables to infant birth weight. This additional analysis diminished the sample size; the women with all three trimester non-fasting glucose levels totalled 92 sets (Table V).

Table VI depicts the results of the analysis when the variables of the second and third trimester were subsequently adjusted for variables in the prior trimester or trimesters. The adjusted second-trimester mean fast-

Median			*Minimum*			*Maximum*		
1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
125	114	105	19	26	5	554	411	301
113	104	90	9	2	40	400	355	297

ing plasma glucose ($p = 0.035$), the adjusted third-trimester mean nonfasting plasma glucose ($p = 0.009$), and the adjusted third-trimester mean glycosylated hemoglobin ($p = 0.018$) levels showed a significant correlation with infant percentile birth weight. Again, this analysis diminished the sample size.

Table VII shows the data from the self-monitored blood glucose diaries. Because the number of observations in the third trimester was lower than the preceding trimesters, we used the diary data from the third trimester to confirm the relationship between glucose and macrosomia seen in the laboratory values. The same observation was found between the fasting and nonfasting values. It is noteworthy that the correlation between the diary values and the laboratory values was excellent ($r = 0.81, p < 0.001$).

Elevated blood glucose variables in the first trimester did correlate significantly with elevated variables in the third trimester (fasting glucose: $r = 0.43, p = 0.0001$; nonfasting glucose: $r = 0.48, p = 0.0001$; glycosylated hemoglobin: $r = 0.68, p = 0.0001$). Thus it was shown that those women who had elevated glucose variables in the first trimester tended to be the same women who had the elevated variables in the third trimester. It is noteworthy that the normal controls also showed a similar relationship between blood glucose values in the first and third trimester (pooled fasting and nonfasting glucose: $r = 0.53$, $p = 0.0002$; glycosylated hemoglobin: $r = 0.23$, $p = 0.01$), although there was no relationship between the normal controls' parameters of glucose control and their infants' birth weight.

When all the analyses were repeated with adjustment for hypertension, renal dysfunction, smoking, ponderal index, and weight gain, the following observations were made:

1. Sixty-one of 323 women had a blood pressure level >140/90 mm Hg at any time during the pregnancy. Macrosomic babies were significantly less likely to be associated with a high blood pressure ($p = 0.004$) (Wilcoxon rank sum test).

2. Twenty-five of 323 women had renal dysfunction, classified as White class F or RF. The lower birth weight babies were more likely to be associated with mothers who were classified as White class F or RF ($p = 0.001$). Macrosomic infants were significantly less likely to be associated with White class F or RF.

3. Forty-nine of 323 women smoked. There was no association between birth weight groups and smoking (p = NS).

4. There was no association between ponderal index and birth weight groups (p = NS).

5. Fifteen of the 323 women gained >20 kg by 32 weeks' gestation. A weight gain in the mother ≥20 kg by week 32 is more likely to be associated with a high birth weight in the infant. All 15 women who gained ≥20 kg had infants whose birth weight was in the top 50th percentile. Seven of these women (47%) had infants in the top 10th percentile for birth weight, the definition of macrosomia.

Comment

The Diabetes in Early Pregnancy Study undertook a prospective analysis of the relationship between maternal glucose control during pregnancy complicated by diabetes and subsequent percentile birth weight. The data indicate that glycemic control in late pregnancy is the strongest metabolic determinant of birth weight, but there is a major contribution from the nonfasting and glycosylated hemoglobin from both prior trimesters.

If elevated glycosylated hemoglobin levels and macrosomia are each mediated by ambient glucose levels, then the nonfasting plasma glucose level appears to contribute more to both the glycosylated hemoglobin and to the risk of macrosomia than the fasting plasma glucose. Early studies of glycosylated hemoglobin documented that hemoglobin A_{Ic} levels correlated better with the peak plasma glucose or the area under the curve on a glucose tolerance test than with the fasting glucose value.[24] Our study shows that peak glucose levels during the day are more predictive of the infant birth weight than the lowest blood glucose level of the day (the fasting). Our finding that the nonfasting glucose level correlates with infant birth weight is consistent with the Pedersen hypothesis[2] in that glucose is still implicated as a causal factor.

Fetal growth is obviously complex and it is possible that metabolic influences on growth are mediated by growth factors or fuels that were not measured.[4] Perhaps we could have obtained a more detailed assessment of the influence of maternal metabolic control on fetal growth if detailed characterization of additional maternal fuels and direct assessment of fetal β-cell function has been feasible. Furthermore, other factors

have been reported to affect fetal growth (e.g., maternal weight, smoking, renal dysfunction, or hypertension)[13] but controlling for these variables did not appreciably alter the significant correlations we found between maternal glycemic control and infant birth weight.

We were intrigued by the correlations between first- and second-trimester indices of glycemic control with birth weight because adipocyte proliferation and the storage of lipids in the fetus occur primarily in the third trimester. The logistic regression analysis did assign greatest significance to glycemic control in the third trimester, although the analysis did diminish our sample size. This could mean that the correlations we observed with birth weight and glycemic control in the first and second trimesters were merely a reflection of the fact that glycemic control was strongly intercorrelated throughout gestation. However, early and late metabolic events may be linked by other mechanisms as well. Thus insulin has been detected in the human fetal pancreas by 10 to 12 weeks[25] and in fetal blood by 11 weeks.[26] Secretion of insulin has been observed in vitro in response to ionic and metabolic stimuli with islets or pancreatic tissue from fetuses in the late phase of the first and early phase of the second trimester.[27, 28] Furthermore, pancreases from fetuses of diabetic mothers have increased β-cell mass and secrete more insulin in vitro[29] by 20 weeks' gestation compared with those from nondiabetic mothers. Thus it is possible that the magnitude of hyperglycemia during early and midgestation may influence the extent of β-cell hyperfunction in later pregnancy and set the stage for the development of macrosomia in the face of continuing hyperglycemia in the third trimester.

Regardless of the mediating factors, our data also indicate that monitoring only fasting and premeal glucose concentrations, which reflect the lowest glucose levels of the day, does not provide an adequate indication of overall metabolic control and the risk of macrosomia. If women with diabetes who are at risk of macrosomic infants are to be identified early and have their treatment altered to improve metabolic control, optimum surveillance should include measurements of glycosylated hemoglobin and nonfasting (postprandial) blood glucose concentrations throughout gestation.

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Association between hypothermia and mortality rate of premature infants—Revisited

Joëlle Hazan, MD, Urs Maag, PhD, and Philippe Chessex, MD
Montreal, Quebec, Canada

The incidence of hypothermia (axillary temperature <35° C) on admission of an infant to a neonatal intensive care unit was retrospectively analyzed in 559 very-low-birth-weight (<1500 gm) newborn infants. The smaller infants were at greater risk of hypothermia. Only in the larger neonates was mortality related to hypothermia, which suggests that this known association bears little cause-effect relationship. (Am J Obstet Gynecol 1991;164:111-2.)

Key words: Prematurity, body temperature, hypothermia, mortality

Very-low-birth-weight preterm newborn infants are prone to thermoregulatory problems because they have important sources of heat loss coupled with a limited supply of brown fat for nonshivering thermogenesis. Alterations in temperature homeostasis can have serious consequences on the mortality rate of preterm infants.[1] With the survival of smaller infants, it was deemed of interest to evaluate the incidence of hypothermia on admission, as an indicator of the quality of perinatal care.

Methods

Appropriate-for-gestational-age inborn and outborn infants weighing between 750 and 1500 gm, who were admitted to the Neonatal Intensive Care Unit of Hôpital Sainte-Justine during two 2-year periods (1980-1981 and 1984-1985) entered this retrospective study. An analysis of the infant's chart was conducted to evaluate axillary temperature on admission, duration of hospitalization, and survival rate. Hypothermia was defined a priori as a temperature <35° C. Duration of hospitalization was defined as the total number of days spent in the unit before death, discharge, or retransfer to the referring hospital. Infants transported after 12 hours of age, as well as stillbirths and infants with chromosomal anomalies or malformations, were excluded. The population was divided into three subgroups according to birth weight: group 1, 750 to 999 gm (n = 128); group 2, 1000 to 1249 gm (n = 172); group 3, 1250 to 1500 gm (n = 259).

In the delivery room, inborn infants were placed under a radiant heater, on a warm sheet, until the skin was dried thoroughly. After resuscitation, the neonates were transported for stabilization to the neonatal intensive care unit, adjacent to the delivery room. The outborn infants were transported by a team consisting of one neonatal nurse and one respiratory therapist. Before transport, the infant's condition was assessed; resuscitation and stabilization procedures were carried out in the referring hospital. The temperature of the transport incubator (model TI100, Air Shields) was preset according to the infant's birth weight: 750 to 999 gm, 40° C, 1000 to 1249 gm, 38° C, 1250 to 1500 gm, 36° C. On admission in the neonatal unit, axillary temperature was measured by the nurses with a mercury thermometer to the closest 0.1 ° C. Axillary instead of rectal temperature was monitored to avoid trauma.

Statistical comparisons were performed by Student's t test for the parametric measurements; differences in frequencies of observations were analyzed by χ^2, with

From the Perinatal Service, Hôpital Sainte-Justine, and the Department of Pediatrics and the Department of Computer Science and Operations Research, University of Montreal.
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Reprint requests: Philippe Chessex, MD, Centre de Recherche, Hôpital Sainte-Justine, 3175 Chemin Côte Sainte-Catherine, Montreal, Quebec, Canada H3T 1C5.
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Table I. Incidence of hypothermia (temperature <35° C) among survivors and deceased infants

Group	n	Hypothermia	Hypothermia among survivors		Hypothermia among deceased infants	
			No.	%	No.	%
750-999 gm	128	30	13/61	21	17/67	25
1000-1249 gm	172	20	11/138	6	9/34	26*
1250-1500 gm	259	16	9/227	4	7/32	22*

*$p < 0.01$ for comparison of incidence of hypothermia between survivors and deceased infants.

Yates' correction for continuity. Incidences of hypothermia were compared between survivors and deceased infants for the entire populations (750 to 1500 gm) of inborn and outborn infants. For multiple comparisons the Bonferroni method was used. A probability value <0.05 was considered significant.

Results

The birth weights, gestational ages, and Apgar scores at 5 minutes were not significantly different between the inborn and outborn populations. Those infants in the smallest birth weight group (750 to 999 gm) had the lowest ($p < 0.01$) temperature on arrival in the unit, in the outborn as well as the inborn populations (mean ± SD 35.1° ± 0.8° and 35.3° ± 0.9° C), compared with the 1000 to 1249 gm group (35.8° ± 0.8° and 35.5° ± 0.7° C), as well as with the 1250 to 1500 gm newborn infants (36.0° ± 0.9° and 35.7° ± 0.6° C).

There was no difference in the incidence of hypothermia between 1980-1981 and 1984-1985. Therefore data on hypothermia for those two periods were combined. Furthermore, there was no difference between inborn and outborn populations in the overall incidence of hypothermia (47/407 or 11.5% vs 19/152 or 12.5%) or in the incidence of hypothermia among survivors (25/312 or 8% vs 8/114 or 7%) or deceased infants (25/95 or 23% vs 11/38 or 29%). Therefore both populations were combined in Table I. The incidence of hypothermia was statistically greater ($p < 0.001$) in the smallest infants (30/128 or 23%) compared with the 1250 to 1500 gm group (16/259 or 6%). Except for the infants in the smallest birth weight group, hypothermia was significantly more frequent in the subgroup of infants who died. Hypothermia did not affect the length of hospitalization (750 to 999 gm, 97 ± 31 vs 89 ± 43 days; 1000 to 1249 gm, 59 ± 19 vs 60 ± 34 days; 1250 to 1499 gm, 41 ± 12 vs 44 ± 29 days).

Comment

Cold stress or hypothermia has been reported for rectal temperatures ranging from 32° to 36° C. Mortality among premature infants has been shown to be significantly greater when rectal temperature on arrival in the hospital was <34° C.[2] In this study a similar association was found in the larger infants despite a higher axillary temperature (<35° C); axillary temperature has been shown to correlate closely with rectal temperature.

Although larger newborn infants are able to react to a cold stimulus by increasing metabolic rate, it is hardly surprising that the sicker preterm infants do not have temperature regulated as well and that consequently neonatal mortality could be related to hypothermia on arrival in the hospital. However, numerous confounding factors could account for the fact that this is merely an association, with little cause-effect relationship. Indeed such an association was not found in the smallest infants (<1000 gm), who had the lowest mean temperature and the highest incidence of hypothermia on arrival in the unit. This could be explained by the fact that the thermogenic capacity of the very small premature infant, whether sick or not, can be rapidly insufficient to prevent a fall in body temperature. Therefore in the smallest preterm infants one has to be careful about attributing poor neonatal outcome to hypothermia on admission to the nursery.

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The accuracy of clinical findings and laparoscopy in pelvic inflammatory disease

John Sellors, MD,[a, c, f] James Mahony, PhD,[d] Charles Goldsmith, PhD,[f] Darlyne Rath, BScN,[f] Rajinder Mander, MD,[b] Barry Hunter, MD,[b] Clifford Taylor, MD,[b] David Groves, PhD,[d] Harry Richardson, MD,[d] and Max Chernesky, PhD[e]

Burlington and Hamilton, Ontario, Canada

The accuracy of clinical diagnosis for pelvic inflammatory disease was determined in 95 women who presented with pelvic pain to primary care physicians and then were referred to gynecologists. Laparoscopy or laparotomy with endometrial biopsy and fimbrial minibiopsy revealed that prevalence of pelvic inflammatory was 46% (44/95) and positive and negative predictive values of gynecologists were 74% (23/31) and 67% (43/64) ($p = 0.0002$). If histopathologic diagnosis was the standard, clinical accuracies of the gynecologists were no better than chance ($p = 0.43$), suggesting an expectation bias for visual diagnosis. Laparoscopy had a sensitivity of 50% (12/24) and a specificity of 80% (40/50) for salpingitis if the standard was fimbrial histopathologic diagnosis ($p = 0.01$). These results support the routine use of laparoscopy, supplemented when negative by endometrial and fimbrial minibiopsy, to accurately diagnose pelvic inflammatory disease. (Am J Obstet Gynecol 1991;164:113-20.)

Key words: Pelvic inflammatory disease, laparoscopy, diagnostic errors, biopsy, routine diagnostic tests

Jacobson[1] was the first to measure the accuracy of clinical diagnoses in women hospitalized for pelvic pain. The positive predictive value of a clinical diagnosis of salpingitis was 70% and the negative predictive value of other clinical diagnoses was 79% when the standard of diagnosis was laparoscopy or laparotomy. Clinical signs of salpingitis such as cervical motion and adnexal tenderness have been evaluated for sensitivity and positive predictive value[1-3] but not for their specificity and negative predictive value. The probability of disease in a patient cannot be estimated without knowledge of the specificity of a sign, even if the sensitivity and prevalence of disease are known.[1] A final concern is the adoption of laparoscopy as the diagnostic standard of salpingitis without validation.[5]

Much research has been done on women hospitalized with suspected pelvic inflammatory disease despite the fact that most cases are managed outside of hospitals.[6] We analyzed the clinical, laboratory, operative, and histopathologic findings of women who presented to primary care physicians because of pelvic pain.

From the Departments of Family Medicine[a] and Obstetrics and Gynecology,[b] Joseph Brant Memorial Hospital, Burlington, and the Departments of Family Medicine,[c] Pathology,[d] Pediatrics,[e] and Clinical Epidemiology and Biostatistics,[f] McMaster University, Hamilton.
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Reprint requests: John W. Sellors, MD, 15 Mountain Ave. S., Suite 201, Stoney Creek, Ontario, Canada L8G 2V6.
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Material and methods

Sample. In Burlington, Ontario, consecutive women with pelvic pain, presenting to 65 family physicians and the six casualty officers at the emergency department of the hospital, were referred to one of three gynecologists for laparoscopy or laparotomy if the primary care physician considered the pain to represent pelvic inflammatory disease or another important condition (e.g., ectopic pregnancy, appendicitis). Diagnoses were made as usual and there were no preset criteria insisted on for the diagnosis of pelvic inflammatory disease (see Comment). Primary care physicians and gynecologists were allowed to use any diagnostic tests available to them before they recorded their preoperative diagnoses. Eligibility criteria were that the women had to be premenopausal and not known to be pregnant, no antimicrobial treatment, abortion, or abdominal surgery had occurred in the previous 6 weeks, the uterus and at least one fallopian tube had to be present, the pain had not been present for more than 2 months, and they had to be fit for surgery. Of the 95 women who signed a consent form approved by the McMaster University Ethics Committee and underwent surgical exploration, 63% presented to the primary care physician in the office, 33% came to the emergency department and 4% were seen on the wards. Pelvic pain was assessed as mild, moderate, or severe in 3%, 63%, and 34%, respectively, and the mean ± SD delay from

patient entry to operation was 1.7 ± 5.2 days. Another 44 women (one of whom refused to give consent) were thought by the gynecologist not to have serious pathologic conditions, were not entered, and were observed only.

Operative procedure. With the patients under general anesthesia, a sample of vaginal fluid and four endocervical swabs were obtained for microbiologic studies. After the cervix had been cleansed with povidone-iodine solution, biopsy of the endometrium was performed with a Novak suction curette in 92% (87/95). Laparoscopy (carbon dioxide pneumoperitoneum with the two-puncture technique), laparotomy, or both were carried out in standard fashion in 73, 14, and 8 patients, respectively. Fimbrial minibiopsies (approximately 1 mm^3) were done with laparoscopy scissors in 78% (74/95). Three endotubal swabs were obtained or a fallopian tube was aspirated as previously described,[7] and a sample of cul-de-sac fluid was aspirated for bacteriologic studies. The gynecologist recorded the operative findings and postoperative diagnosis.

Laboratory methods. Bacterial vaginosis was diagnosed from vaginal fluid if at least three of four signs were present (thin and homogeneous appearance, pH >4.5, smell of volatile amines after the addition of 10% potassium hydroxide, or presence of clue cells on wet mount examination) or if a Gram-stained smear showed typical changes.[8] Endocervical swabs were inserted serially and rotated to obtain samples in the following order: the first for *Neisseria gonorrhoeae* culture[9] and detection of purulent cervicitis[10] and intracellular gram-negative diplococci on Gram-stained smear, the second for aerobic[11] and anaerobic[12] cultures, the third for mycoplasma cultures[13] (*Mycoplasma hominis* and *Ureaplasma urealyticum*), and the fourth for *Chlamydia trachomatis* by cell culture[14] (after cervical mucus had been removed with a cotton swab). Similarly, aspirates of cul-de-sac fluid and the three endotubal swabs were cultured for *N. gonorrhoeae,* aerobes, anaerobes, mycoplasmas and *C. trachomatis*. Specimens were inoculated in the operating room to optimize sensitivity of the bacteriologic methods. After inoculation anaerobic plates were placed in the Marion Bio Bag (Nordic Scientific, Kirkland, Quebec) anaerobic transport system and transferred into the anaerobic chamber without opening the Bio Bag container.

Midstream urine samples were sent for urinalysis, culture, and testing (sensitivity 500 IU/L) for human chorionic gonadotropin β-subunit (β-hCG) (Neo Planotest Duoclon, Organon Teknika, Scarborough, Ontario). Preoperative blood samples were obtained for complete blood count, erythrocyte sedimentation rate, C-reactive protein,[15] (normal value <18 mg/L), and radioimmunoassay for β-hCG[16] (positive, >5 IU/L). Results of these tests were not available preoperatively unless an attending physician had ordered them. In preoperative and convalescent (2 months after operation) serum samples, *C. trachomatis* immunoglobulin G antibody was measured by the microimmunofluorescence technique of Wang et al.[17] and the criterion for a serologic diagnosis of chlamydial infection was seroconversion or a fourfold or greater change in titers. Preoperative sera were screened for immunoglobulin A antibody to *C. trachomatis* by a slide immunoperoxidase test (Ipazyme; Sargon Diagnostics, Ltd; Ramat Gan, Israel) with a single dilution of 1:16. Biopsy specimens were fixed in 10% formalin and paraffin-embedded sections were stained: one from each patient by hematoxylin and eosin and one with the methyl green-pyronin method for plasma cell identification.[18]

Definitions of pelvic inflammatory disease, endometritis, and salpingitis. Pelvic inflammatory disease was assessed by either visual signs of salpingitis or histopathologic evidence from fimbrial or endometrial biopsy specimens (ALL-PID) or, alternatively, by histopathologic evidence only (PATH-PID). The minimal signs for a gynecologist to make a visual diagnosis of salpingitis were abnormal erythema and edema of the fallopian tubes. The presence of spontaneous or expressible inflammatory exudate from the fallopian tube(s) was not a prerequisite if the gynecologist thought that salpingitis was definitely present visually. Bacterial growth from an upper genital tract specimen was never present without visual and histopathologic evidence of salpingitis. Tissue sections were examined independently by three pathologists who were given only the patient's age and number of days since her last menstrual period. To avoid overdiagnosis of endometritis and salpingitis, a consensus was necessary among the three pathologists that plasma cells, lymphoid aggregates (perivascular or subepithelial), or polymorphonuclear leukocytes in the tissue sections were diagnostic of pelvic inflammatory disease. Inflammation was graded as acute if there was a predominance of polymorphonuclear leukocytes and chronic if plasma cells or lymphoid aggregates prevailed. Subacute inflammation showed both acute and chronic features. Plasma cells were seen in 88% (15/17) of sections showing endometritis and 75% (18/24) of those showing salpingitis. To test the validity of histopathologic diagnosis as a criterion, observer agreement studies with the three pathologists were conducted on a random (with a random number table) sample of half of the endometrial and fimbrial sections. Between pairs of pathologists, simple agreement varied from 80% to 91% and agreement beyond that due to chance (κ coefficient[19]) varied from 0.49 to 0.80, indicating fair to excellent agreement.[20]

Statistical methods. We chose a clinically impor-

Table I. Performance of clinical diagnosis by gynecologists and primary care physicians for presence or absence of pelvic inflammatory disease when the standard of positivity was ALL-PID (n = 95) or PATH-PID (n = 92)

Comparison	Sensitivity		Specificity		PPV	NPV	p Value*
	%	No.	%	No.			
GYN vs ALL-PID	52.3	23/44	84.3	43/51	74.2	67.2	0.0004†
GYN vs PATH-PID	40.6	13/32	70.0	42/60	41.9	68.9	0.426
PCP vs ALL-PID	61.4	27/44	52.9	27/51	52.9	61.4	0.235
PCP vs PATH-PID	59.4	19/32	46.7	28/60	37.3	68.3	0.738

PPV, Positive predictive value; *NPV*, negative predictive value; *GYN*, gynecologists; *PCP*, primary care physicians.
*From χ^2 test.
†Statistically significant.

tant difference (positive predictive value minus false-negative rate) of 30% for the purpose of sample size estimation. If the difference was found to be ≤30%, there was definite cause for concern about the ability of clinical examination to detect pelvic inflammatory disease. A significance level, α, was chosen to be 0.05 (one-tailed) and the probability of a type II error, β, was chosen to be 0.10 with a χ^2 test. A sample size of 95 women sufficed since recruitment ratios for the clinical diagnoses of non–pelvic inflammatory disease/pelvic inflammatory disease were 45:50 and 64:31 for primary care physicians and gynecologists. Clinical accuracy was calculated by dividing the sum of the true-positive and true-negative results by the total number of patients. Since there were repeated measurements (two) on each of the patients, one by a primary care physician and another by a gynecologist, McNemar's test (with Yates' correction) for matched pairs was used to compare primary care physicians with gynecologists. Two-tailed p values were computed and denoted significant if they were $<\alpha$. The χ^2 statistic with Yates' continuity correction or Fisher's exact test was used to assess the significance of any associations. Power estimates for nonsignificant differences were from standard tables.[21] The optimal IgG antibody titer for prediction of pelvic inflammatory disease was determined from a receiver-operating-characteristic curve.[4]

Results

Accuracy of clinical diagnosis. The diagnostic performances of gynecologists and primary care physicians, shown in Table I, were no better than those achievable by chance alone, except when gynecologists were compared with ALL-PID (p = 0.0004). The poor performances of the clinicians are probably not due to inadequate sample size since the statistical power of this study to detect a clinical accuracy of 85% (78/92) for PATH-PID by gynecologists, instead of the observed 60% (55/92), was 0.97 (one-tailed α = 0.05) (for this calculation accuracy is the sum of true-positive and true-negative values divided by total number of patients). Among the 95 women assessed with ALL-PID used as the standard of positivity, there were 23 true-positive clinical diagnoses by gynecologists. The false-negative clinical diagnoses by gynecologists were: tubal pregnancy (n = 5), no cause found (n = 4), ovarian cyst (n = 4), hemorrhagic corpus luteum cyst (n = 2), endometriosis (n = 2), appendicitis (n = 2), pyelonephritis (n = 1), and irritable bowel syndrome (n = 1). Of the eight women with false-positive clinical diagnoses of pelvic inflammatory disease, no cause was found in five and endometriosis was diagnosed in three. Women with true clinically negative results had the following final diagnoses: no cause found (n = 19), ovarian cyst (n = 9), appendicitis (n = 5), tubal pregnancy (n = 3), endometriosis (n = 3), irritable bowel syndrome (n = 1), Crohn's disease (n = 1), retrograde menstruation (n = 1), and pelvic congestion syndrome (n = 1). In conjunction with confirmed pelvic inflammatory disease two women had periappendicitis[22] and two others had perihepatitis.[23]

For each case the clinical diagnoses of primary care physicians and gynecologists were compared by matched pair analysis on sensitivity, specificity, positive predictive value, and negative predictive value (Table II). The only significant differences were in specificity and negative predictive value, with gynecologists being superior.

Histopathologic and visual diagnosis of pelvic inflammatory disease. With additional evidence from endometrial and fimbrial biopsy specimens in women with negative visual findings, the prevalence of confirmed pelvic inflammatory disease increased from 30% (28/95) with visual diagnosis alone to 46% (44/95). The diagnostic performances of visual signs and endometrial biopsy are shown in Table III. Endometrial biopsy findings were positive in one third of women with confirmed salpingitis and four patients with pelvic inflammatory disease had endometritis without salpingitis. Compared to fimbrial minibiopsy, visual diagnosis (by

Table II. Comparison by matched pair analysis of diagnostic performances of primary care physicians and gynecologists when the standard of positivity was ALL-PID or PATH-PID

	PCP (%)	*GYN (%)*	χ^{2*}	*p Value (2-tailed)*
ALL-PID (visual or histologic criteria, n = 95)				
SENS	61	52	0.75	0.39
SPEC	53	84	14.06	0.0002†
PPV	53	74	0.75	0.39
NPV	61	67	14.06	0.0002†
PATH-PID (histologic criteria only, n = 92)				
SENS	59	41	2.50	0.11
SPEC	47	70	9.39	0.002†
PPV	37	42	2.50	0.11
NPV	68	69	9.39	0.002†

PCP, Primary care physicians; *GYN*, gynecologists; *SENS*, sensitivity; *SPEC*, specificity; *PPV*, positive predictive value; *NPV*, negative predictive value.

*McNemar's χ^2.

†Statistically significant.

Table III. Performance of visual diagnosis and endometrial biopsy for presence or absence of pelvic inflammatory disease when the standard of positivity was fimbrial histopathologic diagnosis, PATH-PID, or at least one of fimbrial histopathologic diagnosis or visual signs

Comparison	*Sensitivity*		*Specificity*		*PPV*	*NPV*	*p Value†*
	%	*No.*	*%*	*No.*			
Visual signs† vs fimbrial biopsy‡	50.0	12/24	80.0	40/50	54.5	76.9	0.017‡
Visual signs vs PATH-PID	48.4	15/31	78.7	48/61	53.6	75.0	0.015‡
Endometrial biopsy vs fimbrial biopsy	39.1	9/23	91.1	41/45	69.2	74.5	0.007‡
Endometrial biopsy vs fimbrial biopsy or visual signs	33.3	13/39	91.5	43/47	76.5	62.3	0.009‡

*From χ^2 test.

†85% (81/95) of women were assessed by laparoscopy, and 15% by laparotomy.

‡Statistically significant.

laparoscopy in 85% of patients) missed half of those with histopathologically confirmed salpingitis. Chronic and acute salpingitis was present in 83% (20/24) and 17% (4/24), respectively, of the positive fimbrial minibiopsy specimens. Endometrial histologic examination showed subacute inflammation in 82% (14/17) and chronic inflammation in 18% (3/17).

The confirmation rates by fimbrial histopathologic diagnosis for groups of laparoscopic visual signs are shown in Table IV. Twelve women with only tubal erythema and edema and no purulent exudate would have been negative according to conventional criteria,[2,5] but the rate of confirmation by fimbrial histopathologic diagnosis was the same as for the "mild" category.[5] Eleven other women had laparoscopically negative findings for salpingitis but fimbrial histopathologic diagnosis showed two with acute salpingitis and nine with chronic salpingitis (plasma cells in eight). The laparoscopic criteria recommended by Hager et al.[5] would have missed 58% (14/24) with histologically confirmed salpingitis.

Ectopic pregnancy. Fimbrial histopathologic examination, at a site distant from the ectopic implantation in the seven women with confirmed ectopic pregnancy, showed that three had chronic salpingitis and one had acute salpingitis, which was also visually obvious. Assays for β-hCG in urine and sera had sensitivities for ectopic pregnancy of 43% (3/7) and 100% (7/7), respectively. The five patients in the false-negative category with clinical diagnosis of ectopic pregnancy had salpingitis confirmed by fimbrial histopathologic diagnosis and four of them also had ectopic pregnancy.

Oral contraception and severity of pelvic inflammatory disease. The prevalence of visually mild salpingitis (including those without purulent tubal exudate) or false-negative laparoscopic results (positive

fimbrial histopathologic diagnosis) among women with salpingitis who used oral contraceptives was 79% (11/14) versus 70% (21/30) in all others with salpingitis ($p = 0.42$). The power of this sample size to detect a 40% (12/30) prevalence of less severe disease in those not using oral contraceptives was >0.75 (one-tailed $\alpha = 0.05$).

Predictors of pelvic inflammatory disease. The mean ± SD age of the 95 women was 25.1 ± 6.6 years, 54% were single, and all but one were white. Nulliparous women constituted 58% of the group, 25% were not using contraception, 34% were using oral contraceptives, 8% were using an intrauterine contraceptive device (IUD), and 30% reported IUD use in the past. The mean ± SD age at beginning coitus was 16.8 ± 2.3 years, mean ± SD number of sexual partners in the preceding 12 months was 1.3 ± 0.8, and there was history of sexually transmitted disease and pelvic inflammatory disease in 12% and 30%. A change of sexual partner in the preceding 2 months was reported by 24%. Data on oral sex, douching, and frequency of sexual intercourse were not obtained. All of the demographic, behavioral, clinical, investigational, and operative findings collected by the gynecologists were compared between those with and those without pelvic inflammatory disease to detect significant differences. Variables that had statistically significant associations with the presence of confirmed pelvic inflammatory disease are shown in Table V. There were no significant associations for the rest of the variables, including severity of pain, history of fever, chills, irregular bleeding, urethritis symptoms, nausea, vomiting, diarrhea, increased body temperature, cervical motion tenderness, adnexal tenderness, uterine tenderness, palpable adnexal mass, abnormal vaginal discharge, or presence of bacterial vaginosis or cervicitis (microscopic criterion). The study had a statistical power of at least 0.90 to detect a 30% difference in any of these variables.

IgG antibody to *C. trachomatis* at a titer of ≥1:8 was found in 63% (27/43) of women with ALL-PID and 43% (21/49) of those without ALL-PID ($p = 0.09$) (three acute sera missing). Similarly, IgG antibody was present in 71% (22/31) of women with PATH-PID and 43% (25/58) of those without it ($p = 0.02$). At a dilution of 1:256, 16% (7/43) of those with ALL-PID and none (0/49) of those without it had detectable IgG antibody ($p = 0.004$). Nineteen percent (6/31) of those with PATH-PID and 2% (1/58) of those without it had positive results at a titer of 1:256 ($p = 0.007$). The IgG titer of 1:128 (Table V) was chosen (from receiver-operating-characteristic curves of the dilutions from 0 to ≥1:256) to be the optimal dilution for predicting pelvic inflammatory disease, considering the false-positive and true-positive rates. IgA serum antibodies to *C. trachomatis* were more common in women with pelvic inflammatory disease, whether as ALL-PID ($p = 0.001$) or PATH-PID ($p = 0.03$) (Table V).

Table IV. Visual signs of inflammation and corresponding histopathologic confirmation rates from fimbrial minibiopsies in 39 women with histologic or visual evidence of salpingitis who had laparoscopy performed*

Visual signs†	*Histologically confirmed (%)*	*No. positive/No. biopsies*
Severe	88.9	8/9
Moderate	100.0	1/1
Mild‡	25.0	1/4
Tubal edema and erythema only§	25.0	3/12
Nondiagnostic	100.0	11/11
TOTAL		24/37

*One additional woman with salpingitis had laparotomy only.

†Severe, moderate, and mild refer to laparoscopic classification proposed by Hager et al. whereby severe includes pyosalpinx, pelvic inflammatory complex, or abscess; moderate includes gross purulent material, marked tubal edema, and erythema; and mild includes erythema, edema, and exudate that might require tubal manipulation for expression.

‡Two women with mild salpingitis did not undergo fimbrial minibiopsy.

§No purulent material could be demonstrated with tubal manipulation.

Microbiologic results. *U. urealyticum* was isolated from the endocervix in 48% (21/44) and 28% (14/51) of women with and without ALL-PID ($p = 0.04$). *C. trachomatis* was isolated from the endocervix of 11% (5/44) and 2% (1/51) of those with and without ALL-PID ($p = 0.07$) and *N. gonorrhoeae* from the endocervix of one woman in each group ($p = 1.00$). Recovery of bacteria from fallopian tube or cul-de-sac specimens occurred in 11% (5/44) of patients with ALL-PID: *C. trachomatis* was found in two women who also had positive findings from the endocervix; *Staphylococcus aureus, U. urealyticum,* and an unidentified species of *Eubacterium,* respectively, were found in the other three. Convalescent sera were available from 84% (37/44) of the women with ALL-PID, 10 of whom showed seroconversion or a fourfold or greater change in IgG antibody titers to *C. trachomatis,* versus two of 38 without ALL-PID ($p = 0.01$). Convalescent serum was not obtainable from one of the women whose fallopian tube specimen was positive in chlamydia culture. The prevalence of chlamydial pelvic inflammatory disease, demonstrated by fallopian tube culture or paired serologic test results, was 25% (11/44).

Comment

These results are generalizable to a primary care sample of women with moderate to severe pelvic pain. For

Table V. Variables with statistically significant differences between those with and without pelvic inflammatory disease defined by two different gold standards

	ALL-PID			*PATH-PID*		
	Present	*Absent*	*p Value*	*Present*	*Absent*	*p Value*
Demography						
Divorced	5/44	0/51	0.01*	5/32	0/60	0.002*
Homemaker	12/44	5/50	0.07	12/32	5/59	0.0007*
History						
Days of pain†	14.8 ± 20.7	26.5 ± 31.2	0.007*	11.4 ± 15.8	27.1 ± 31.0	<0.0001*
Gravida >3	7/44	1/51	0.02*	8/32	2/60	0.002*
History of abortion	18/44	10/51	0.02*	16/32	16/60	0.03*
Current IUD use	7/44	1/51	0.02*	5/32	2/60	0.03*
History of STD	8/44	3/51	0.12	7/32	3/60	0.03*
Signs						
Lower abdominal guarding	28/44	19/51	0.02*	19/32	27/60	0.27
Cervicitis‡	12/44	8/51	0.26	8/32	4/60	0.01*
Investigations						
ESR (mm/hr)†	21.2 ± 21.2	12.8 ± 14.1	0.006*	24.8 ± 22.7	12.2 ± 13.3	0.001*
CRP (mg/L)†	59.4 ± 93.4	20.1 ± 27.6	<0.0001*	63.1 ± 81.6	24.8 ± 58.7	0.03*
WBC ($\times 10^9$/L)†	10.4 ± 3.5	8.9 ± 4.2	0.20	10.6 ± 3.1	9.1 ± 4.3	0.04*
IgG chlamydia antibody§	15/43	3/49	0.001*	13/31	5/58	0.0006*
IgA chlamydia antibody‖	14/44	3/51	0.001*	10/32	7/58	0.03*

STD, Sexually transmitted diseases; *ESR,* erythrocyte sedimentation rate; *CRP,* C-reactive protein; *WBC,* white blood cell count.
*Statistically significant.
†Values are mean ± SD.
‡By visual criteria.
§Serum titer ≥1:128.
‖Serum titer ≥1:16.

ALL-PID the accuracy of our gynecologists was similar to that found by Jacobson[1] in hospitalized women without benefit of endometrial and fimbrial histopathologic diagnosis. The only statistically significant clinical accuracy, that of gynecologists compared with ALL-PID, may have been due to expectation bias[24] since the same gynecologists performed the visual examinations after their clinical assessments. Logistically, this was unavoidable but we anticipated the potential problem by including blind assessment of histopathologic diagnosis. With PATH-PID the accuracy of gynecologists became similar to that of primary care physicians. The sample size had excellent statistical power to show reasonable clinical accuracies if they existed.

Inadequate criteria for visual and histopathologic diagnoses of pelvic inflammatory disease might be suspected as the reason for poor agreement between clinical, visual, and histopathologic assessments. From experience, our gynecologists felt confident that women with both erythema and edema of the fallopian tubes could have mild salpingitis, despite the absence of tubal exudate. Such women would have been diagnosed as negative for salpingitis by the criteria suggested by Jacobson and Westrom,[2] but they had exactly the same histopathologic confirmation rate as that observed in patients with tubal erythema, edema, and exudate. It appears that the visual diagnosis of mild salpingitis was insensitive if tubal exudate was a requirement. In women without these minimal criteria, false-negative laparoscopic results are an acknowledged problem,[5, 25-27] hypothetically because of endosalpingitis. Resolving clinical disease in which histopathologic resolution is lagging behind is another possibility that may have support, since 9 of the 11 women with negative laparoscopic findings and histopathologic evidence of salpingitis had chronic inflammatory changes. However, laparoscopy often revealed salpingitis or bacteria were cultured from the tubes when there were chronic histopathologic changes; prior antimicrobial treatment was an exclusion criterion and most women had illnesses of short duration. Studies that use second-look laparoscopy and fimbrial minibiopsy would be helpful to settle this question. Laparoscopy was not specific for histopathologic evidence of salpingitis possibly because pathologic involvement of the tissues may not have been homogeneous and biopsies or histologic sec-

tions may have missed the abnormal areas. Serosal hyperemia caused by carbonic acid formation from carbon dioxide pneumoperitoneum or the phase of the menstrual cycle may have contributed to visual false-positive results.

Histopathologic criteria for endometritis and salpingitis that define the necessary numbers of plasma cells or lymphocytes in the stroma and polymorphonuclear leukocytes in the mucosa have not been evaluated in the literature. For this reason a consensus by three pathologists, one of whom was an expert in gynecologic pathology (H.R.), was used to provide an unbiased assessment of PATH-PID. The agreement studies suggested that our pathologists were competent and the histopathologic criteria for PATH-PID gave reproducible results. Other investigators have used fimbrial minibiopsy to detect chlamydial infection. Westrom and Mardh[28] took minute specimens of fimbria for culture without any adverse effect on subsequent fertility. Kiviat et al.[7] detected *C. trachomatis* by immunofluorescence staining of fimbrial biopsy specimens, and at second-look laparoscopy DeWilde[29] reported the absence of new fimbrial adhesions 1 week after biopsies had been performed.

Paavonen et al.[30] reported the sensitivity and specificity of endometrial biopsy were 89% and 67% for laparoscopically confirmed pelvic inflammatory disease in women with clinically suspected disease. Our findings showed that endometrial biopsy was less sensitive but more specific, perhaps because of the inclusion of not only the cohort with other clinical diagnoses but also those with histopathologic evidence of salpingitis.

Inclusion in the study of those at risk for pelvic inflammatory disease, but thought to have other causes of pelvic pain, not only changed the estimate of prevalence of pelvic inflammatory disease and demonstrated that one third of these women with pelvic inflammatory disease would have been clinically missed by gynecologists but also revealed the sensitivities and specificities of risk factors and clinical findings. This was the first determination of specificities, since the report by Jacobson and Westrom[2] did not include women who had the clinical impression of the absence of salpingitis confirmed (true clinically negative). Associations between pelvic inflammatory disease and a shorter duration of pain, a history of abortion or sexually transmitted disease, and current IUD use have been reported previously.[26, 31-33] Svensson et al.[34] found a beneficial association between oral contraceptive use and visually milder disease, which we did not confirm. In agreement with previous reports[2, 35-38] elevated levels of several blood tests (erythrocyte sedimentation rate, C-reactive protein, white blood cell count, and serum IgG and IgA antibodies to *C. trachomatis*) were associated with pelvic inflammatory disease (Table V). Only two clinical signs were significantly associated with pelvic inflammatory disease (Table V), and this supports the role of laboratory tests in the clinical assessment of women with pelvic pain.

Serum β-hCG determination should be an essential part of the clinical workup of women with acute pelvic pain.[16] Although serum β-hCG testing performed better than urine testing, it did not discriminate well between those with pelvic inflammatory disease and those with ectopic pregnancy since the two conditions sometimes coexisted in our patients. Histopathologic evidence of chronic salpingitis at a site away from the ectopic implantation has been reported recently,[39, 40] and there is an association between the presence of chlamydial serum antibodies and chronic salpingitis.[39]

With the use of comparable methods our isolation rates of bacteria from intraabdominal sites were different from those of two recent hospital-based North American and Scandinavian studies of laparoscopically confirmed salpingitis.[41, 42] Brunham et al.[39] and Paavonen et al.[42] reported isolation rates of 42% and 71% overall, 4% and 34% for *C. trachomatis*, 18% and 14% for *N. gonorrhoeae*, and 0% and 3% for both microorganisms. The comparatively few infections by other bacteria may reflect sampling of a primary care population of women with fewer previous episodes of pelvic inflammatory disease and milder infections than those in hospital-based studies. Chlamydial pelvic inflammatory disease was detected by isolation from the fallopian tubes or with paired IgG microimmunofluorescence serologic testing in 25% of our patients compared with 8% in the study by Brunham et al.[41] Kiviat et al.[7] have shown that it may be difficult to culture chlamydia from fallopian tube swabs despite demonstration of its antigen in tubal epithelial cells by direct immunofluorescence.

In summary, we found that the clinical diagnosis of the presence or absence of pelvic inflammatory disease is no more accurate than chance when the standard of diagnosis is PATH-PID. Since laparoscopy was insensitive for salpingitis, fimbrial minibiopsy or empirical treatment for pelvic inflammatory disease in women with a negative visual diagnosis may be warranted. Investigations such as serum β-hCG levels, endometrial biopsy, and levels of serum antibody to chlamydia should improve outpatient management in cases of moderate to severe pelvic pain by selecting those that would probably benefit from treatment, laparoscopy, or observation.

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Antiepileptic medication in pregnancy: Late effects on the children's central nervous system development

Michiel C. van der Pol, MD,[a] Mijna Hadders-Algra, MD,[a] Henk J. Huisjes, MD,[b] and Bert C.L. Touwen, MD[a]

Groningen, The Netherlands

In a follow-up study long-term effects of antenatal exposure to two anticonvulsant drugs, phenobarbital and carbamazepine on central nervous system development were evaluated. Children aged 6 to 13 years of epileptic mothers who used phenobarbital (n = 13), carbamazepine (n = 12), phenobarbital plus carbamazepine (n = 12), or no medication (n = 24) during pregnancy were studied. None of the mothers had seizures during pregnancy. A control group carefully matched for confounding variables was selected of children of nonepileptic mothers (n = 61). Minor and major congenital anomalies appeared to be more related to mother's epilepsy than to maternal medication. There were no neurologic differences between the groups. Exposure to phenobarbital was associated with smaller head circumference. An accompanying negative effect of phenobarbital on cognitive development was suggested. Further research on this intriguing phenomenon seems warranted. (Am J Obstet Gynecol 1991;164:121-8.)

Key words: Maternal epilepsy, anticonvulsants, neurologic development, behavioral development, cognitive development

Do antiepileptic drugs taken during pregnancy have a teratogenetic effect on the developing central nervous system? Children exposed to antiepileptic drugs in utero are reported to have developmental delay, subnormal intelligence quotient[1, 2] and language or learning disabilities[3] more often than children of nonepileptic mothers. In children of mothers with a seizure disorder Nelson and Ellenberg[4] found a slightly higher incidence of cerebral palsy and Speidel and Meadow[5] reported a higher frequency of mental subnormality. Other authors found an increase of congenital anomalies[6] and smaller head circumference[2, 7-9] and suggested that this is an effect of exposure to antiepileptic drugs during pregnancy.

The question is whether and to what extent reported abnormalities must be attributed to anticonvulsant therapy or to other factors associated with epilepsy.[10-12] Seizures during pregnancy might be harmful to the fetus (yes[13, 14]; no[15-17]). Epileptic women seem to share a high obstetric risk,[13, 18, 19] a lower socioeconomic status,[15, 20] and higher incidence of family diseases and congenital anomalies[6, 16, 21]; these factors might also have an adverse effect on the offspring.

From the Department of Developmental Neurology[a] and the Department of Obstetrics and Gynaecology,[b] University Hospital.
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Reprint requests: B. C. L. Touwen, MD, Groningen Perinatal Project, University Hospital, Department of Developmental Neurology, P.O. Box 30.001, Groningen, The Netherlands 9700 RB.
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Polytherapy often precludes the analysis of the possible adverse effects of single anticonvulsants. We were able to trace the offspring of small groups of epileptic pregnant women who took either no drugs, phenobarbital, or carbamazepine, or both, and to assess offspring's neurologic, cognitive, and behavioral development at 6 to 13 years of age. A control group was matched for perinatal factors and social class. The study was part of a large follow-up project on perinatal relationships in our University Hospital. No excessive deleterious effects are known of either of the antiepileptic drugs studied. Our question was whether mild neurologic, behavioral, or learning problems may be associated with intrauterine exposure. This makes the present study an explorative one.

Subjects and methods

During the years 1973 to 1981, 132 epileptic mothers were delivered of a live-born infant at the Groningen University Hospital. For the present study, infants were selected who fulfilled the following criteria: (1) documentation of maternal epilepsy by a neurologist; (2) antiepileptic medication: none, phenobarbital, and/or carbamazepine; (3) absence of seizures during pregnancy and delivery; (4) no evidence of intrauterine infection or chromosomal abnormalities; and (5) absence of additional drug exposure.

The selection resulted in a study population of 61 children who had received extensive ante- and perinatal care. Four subgroups were distinguished according to specific antenatal drug exposure: children exposed to phenobarbital only (n = 13); children exposed to carbamazepine only (n = 12); children exposed to

Table I. Number of children examined at follow-up, type of maternal epilepsy; dosage of medication

		Lost to follow-up			*Follow-up achieved (No.)*	*Type of epilepsy*			*Phenobarbital‡*	
	No.	*Death**	*Emigration*	*Refusal*		*Grand mal*	*Temporal psychomotor*	*Other†*	*<150*	*≥150*
Study group										
Phenobarbital	13	—	—	1	12	3	4	5	7	5
Carbamazepine	12	—	1	—	11	5	5	1	—	—
Combination	12	—	—	—	12	7	2	3	3	9
No medication	24	1†	—	1	22	8	6	8	—	—
TOTAL	61	1	1	2	57					
Control group	61	—	1	5	55					

E, Emigration; *R*, Refusal.

*The child died of complications of spina bifida.

†Symptomatic-secondary, nonspecific, unknown.

‡Dosage in milligrams per day.

the combination of phenobarbital and carbamazepine (n = 12); and children not exposed to antiepileptic medication (n = 24).

Information on type of epilepsy and dosage of drug(s) is given in Table I. There were no differences in classification of the epilepsy between the groups. The dosages of antiepileptic drugs were constant throughout pregnancy, except for four mothers of the combination group, whose therapeutic regimen needed a small adaptation during the first trimester.

Standardized data on the blood levels of the antiepileptic drugs were not available but clinical data showed an efficient seizure-suppressing effect.

Control children of nonepileptic mothers were selected from singletons born in the same period. They were matched for their mother's parity and for birth weight, gestational age, sex, age at follow-up, and social class. Data on matching criteria and some additional obstetric data are shown in Table II.

Four children of the study groups and six control children were lost to follow-up; this was either because of death, emigration, or (parental) refusal to cooperate (Table I). The parents who refused cooperation reported that their child was not handicapped. The children's age at follow-up ranged from 6 to 13 years. The examiner was unaware of to which group a child belonged.

A standardized and age-specific neurologic examination was carried out[22] with special emphasis on the presence of minor neurologic dysfunction. The findings were classified as normal, minor neurologic dysfunctions, or abnormal. Abnormal meant the presence of an overt neurologic handicap, such as cerebral palsy. Minor neurologic dysfunctions indicated the presence of a mild deviation (e.g., in posture, muscle tone, coordination, or fine manipulative ability) not resulting in an overtly handicapping condition. The examination included the assessment of skull circumference, body weight, and height. Dutch growth charts according to Gerver (unpublished observation) were used as standards. Attention was paid to the presence of major and so-called minor physical anomalies. The minor anomalies were scored in accordance with Waldrop[23] with exclusion of the items on hair and occipitofrontal circumference. A score >2 was taken as a cutting point. Data on obstetric condition, neonatal course, and interval complications were available. Special attention was paid to diseases with possible involvement of the central nervous system. Interval complications in the first 2 years after birth were distinguished from those that occurred later on inasmuch as there are indications that the brain is more vulnerable during the former period.[24, 25]

Three short standardized Dutch tests for reading,[26] spelling,[27] and arithmetic[28] were applied. Children who scored in the lowest tenth percentile were regarded as poor performers. Test results of children are lacking if they were too young or too old to be assessed with these tests, leading to different numbers of children at different tests. School career was considered nonoptimal if the child attended a school designed for learning disorders or was not in the appropriate grade for age.

Both parents and teachers completed an identical questionnaire on the child's behavior, consisting of questions put in a comparative way: "all children sometimes are . . ., do you regard your/this child as more . . ., or less . . ., or don't see any difference" (Table III). Although subjective these lists provide information of the perception of behavior in two important daily situations: at home and at school. Data on the child's behavior were also obtained with help of the child behavior check list[29] and the children's behavior questionnaire for completion by teachers.[30]

Socioeconomic status was classified as low, lower-middle, and upper-middle, according to the father's occupation.

The study groups were admittedly small. Therefore besides univariate analysis with χ^2, Fisher's exact test,

Carbamazepine‡	
≤400	*>400*
—	—
3	8
3	9
—	—

and the Student t test, multivariate statistics (logistic regression analysis and multiple regression analysis for dichotomized discrete and continuous dependent variables, respectively) were applied. The latter techniques offer an appropriate method to analyze the individual and aggregate effects of two or more variables on an outcome measure. In the present study drug use (carbamazepine, phenobarbital) and the presence of maternal epilepsy were entered as independent variables. The p values were considered significant when they were <0.05 (two-sided).

Results

No significant differences were found in the occurrence of (minor) neurologic dysfunction, either between the control and study groups as a whole, or between the control and specific drug groups (Fig. 1). Two boys were classified as abnormal. One belonged to the carbamazepine group; he turned out to suffer from tuberous sclerosis. The other boy belonged to the control group; he had mild spastic diplegia. Both of them were excluded from further analysis inasmuch as there was no reason to presume a relationship between the mother's use of antiepileptic drugs and the one child's tuberous sclerosis, whereas in the control child drugs were not present. Inclusion would hamper the possibility to answer our specific question because both children had behavioral and learning problems that were directly related to their neurologic disorders.

The phenobarbital group showed a surplus of poor performers on the arithmetic ($p = 0.02$) and spelling ($p = 0.01$) tests (Table IV). The other measurements of cognitive development (school career and reading) were similar in the five groups. No differences between the control and study groups were found with the child behavior check list (parents) and the children's behavior questionnaire (teachers). The two "comparative" behavioral questionnaires (Table III) revealed no significant group differences for the majority of items. However, some noteworthy differences emerged: the teachers reported an excess of short attention span in the phenobarbital group, and the parents noted short attention span more often in the carbamazepine group (Table V).

To validate the findings and to evaluate potential interaction effects of drug use and epilepsy, logistic regression models were applied. For this purpose the four study groups and the control group were pooled. The association between phenobarbital exposure and spelling problems was found again ($p < 0.05$); carbamazepine exposure was associated with a decrease of behavioral problems at school, but this effect seemed to be neutralized by phenobarbital exposure (interaction carbamazepine × phenobarbital, $p < 0.05$). The presence of epilepsy in the mother was not related to any of the neurologic, behavioral, or cognitive outcome measures in the child.

Weight and height distribution at follow-up were comparable in the groups. However, skull circumference in all children of the phenobarbital group was on or below the fiftieth percentile. In the phenobarbital plus carbamazepine group head size distribution also showed a significant shift to the left (Fig. 2, $p = 0.021$, χ^2 goodness of fit). In the other groups such a shift was not found (Fig. 2). Multiple regression analysis of the skull circumference distribution ($r^2 = 0.11$, $p = 0.005$) revealed a significant negative effect of phenobarbital ($p = 0.002$) and a positive effect of carbamazepine ($p = 0.03$) without an interaction between the two. Epilepsy per se had no effect on head size.

The Waldrop score ranged from 0 to 4. Fifty percent, 45%, 25%, and 45% of the children of the phenobarbital, carbamazepine, combination, and no drug study group, respectively, had a Waldrop score >2, compared with 18% of the control group, indicating that minor physical anomalies were more frequent in children of epileptic mothers than in children of nonepileptic mothers (44% versus 18%, $\chi^2 = 8.59$, $p < 0.01$). Major congenital anomalies occurred four times as often in the infants of epileptic mothers than in infants of nonepileptic mothers, but the difference only approached statistical significance ($p = 0.07$, Table VI). The presence of major or minor anomalies was not related to any of the neurodevelopmental outcome parameters.

No dose-response relationship could be shown in any of the areas investigated, but the dosages were appreciably low, ranging from 50 to 200 mg daily for phenobarbital and from 100 to 900 mg a day for carbamazepine.

Comment

The potential teratogenicity of antiepileptic drugs is a complex problem inasmuch as many confounding factors such as polytherapy, the epileptic disorder itself, seizures, and associated obstetric adversities may be present. Many studies focus on morphologic abnormalities as a consequence of intrauterine drug expo-

Table II. Matching criteria and some obstetric data on study and control groups

	PBH $n = 12$	*CBZ* $n = 11$	*PHB + CBZ* $n = 12$	*No medication* $n = 22$	*Control group* $n = 55$
Sex					
Male	4	5	6	12	25
Female	8	6	6	10	30
Age at follow-up (yr)					
6-7	7	6	2	8	21
8-10	3	2	8	9	25
11-13	2	3	2	5	9
Low socioeconomic status	2	2	1	3	9
Parity = 0	5	6	10	11	27
Birth weight (gr)					
Mean	3189	3835	3279	3399	3388
Range	2550-3630	2970-4940	2730-3820	2620-4180	2460-4380
SGA	—	—	—	3	4
Gestational age (wk)					
Range	37-42	39-43	37-42	37-43	37-43
≤37	1	—	1	2	4
pH* ≤7.20	1	1	1	2	5
Apgar scores					
1 minute <7	1	1	—	1	5
3 minute <7	—	—	—	—	2
Obstetric history					
Spontaneous abortion (wk)					
<16	1	1	2	5	6
16-28	—	—	—	—	3
Fetal-neonatal death (wk)					
≤37	—	—	—	—	3
>37	1	2	—	—	—

SGA, Small for gestational age; *PBH*, phenobarbital; *CBZ*, carbamazepine.
*Venous umbilical.

Table III. Behavioral characteristics

Irritability	Easily frightened	Poor concentration
Difficult to handle	Shy, withdrawn	Distractability
Headstrong	Anxious	Short attention span
Temper tantrums	Clumsy	Adaptation
Hyperactive		

sure. Case histories and retrospective studies claim the existence of specific drug syndromes.[6, 31, 32] It has rarely been possible to confirm a relationship between fetal exposure to antiepileptic drugs and congenital anomalies in a prospective design. Several studies indicate that the offspring of epileptic mothers show congenital malformations more often than children of nonepileptic mothers. However, this surplus of malformations is attributed to the presence of the maternal epileptic disorder.[8, 16, 33-35] Our findings corroborate the concept of a probably endogenous origin of congenital abnormalities in children of epileptic mothers rather than a teratogenic cause on the basis of intake of antiepileptic drugs.

The leading question of the present study was not whether fetal exposure to antiepileptic drugs gives rise to morphologic deviations, but whether it might induce functional aberrations, especially of the central nervous system. The majority of the literature suggests an increase of developmental problems such as a developmental delay and mental subnormality in drug-exposed children. Some report a specific drug effect (Hansen et al.,[2] hydantoin; Jones et al.,[32] carbamazepine), others established a negative effect on cognitive abilities of multiple drug therapy.[5] Gaily et al.[36] evaluated intelligence quotient at the age of 5½ years in a group of children prenatally exposed to varying kinds and numbers of antiepileptic drugs (preponderantly phenytoin and carbamazepine). They did not attribute the slight increase of mental deficiency in their study group to fetal exposure of antiepileptic drugs but assumed a genetic connection with the mother's constitution of epilepsy and borderline intelligence.

Our study evaluates possible adverse effects of two specific antiepileptic drugs (phenobarbital, carbamazepine) while controlling for two possible confounders. The influence of maternal epilepsy was assessed by introducing a group of epileptic mothers without drug treatment and a potential disturbing effect of seizures during pregnancy was ruled out by selecting seizure-free cases.

Neither the evaluated drugs nor the maternal epi-

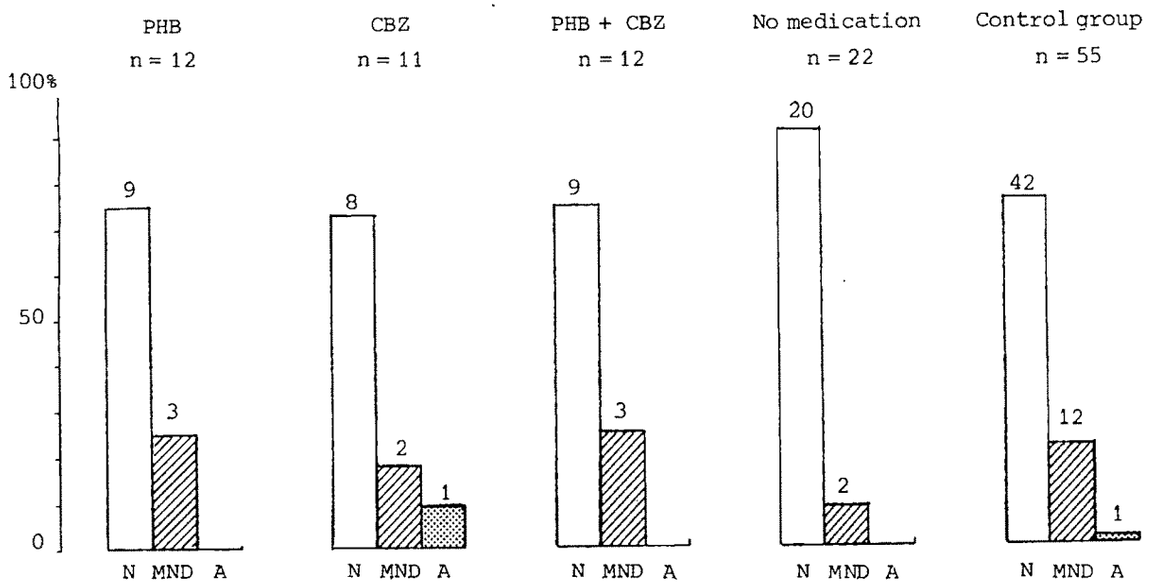


Fig. 1. Neurologic classification at follow-up in study and control groups. *N*, Normal; *MND*, minor neurologic dysfunctions; *A*, abnormal.

Table IV. School career and reading, spelling, and arithmetic skills in study and control groups

	Study groups								*Control group*	
	PHB		*CBZ*		*PHB + CBZ*		*No medicction*			
	N	*n*	*N*	*n*	*N*	*n*	*N*	*n*	*N*	*n*
Nonoptimal school career	12	4	10	—	12	2	22	5	54	10
Poor reading	9	2	9	—	10	3	15	2	43	4
Poor arithmetic	7	3*	8	—	9	1	13	1	37	2
Poor spelling	7	5†	8	—	9	2	13	2	37	7

PHB, Phenobarbital; *CBZ*, carbamazepine.
*Statistically significant differences with control group: $p = 0.02$.
†Statistically significant differences with control group: $p = 0.01$.

Table V. Behavior in study and control groups

	Study groups				
	PHB (n = 12)	*CBZ (n = 10)*	*PHB + CBZ (n = 12)*	*No medication (n = 22)*	*Control group (n = 54)*
School					
Poor concentration	3*	2	1	1	3
Distractable	7	4	1	7	22
Short attention span	7*	2	1	4	14
Home					
Poor concentration	1	1	—	3	1
Distractable	6	4	4	8	15
Short attention span	1	4*	3	5	5

PHB, Phenobarbital; *CBZ*, carbamazepine.
*Statistically significant differences with control group: $p < 0.05$.

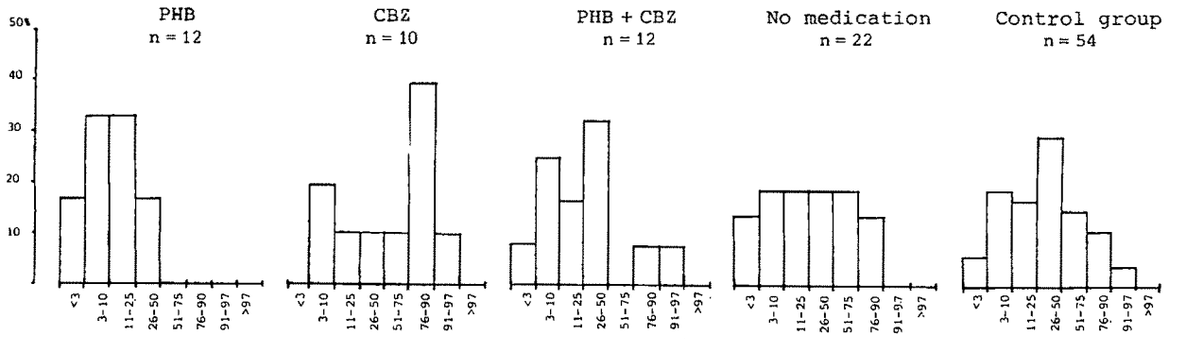


Fig. 2. Distribution of skull circumference in study and control groups (percentiles).

Table VI. Number of major congenital malformations

	PHB (n = 12)	*CBZ (n = 11)*	*PHB + CBZ (n = 12)*	*No drugs (n = 22)*	*Control (n = 55)*
Spina bifida occulta	—	—	—	1*	—
Congenital cataract	—	—	—	1*	—
Digital dysplasia	—	—	—	1	1
Dysplasia of the hips	1	—	—	—	—
Talipes	—	—	1	—	—
Cardiac anomaly	—	1	—	—	1
Abnormal anus	—	—	—	1	—
Hypospadias	—	—	1	—	—

PHB, Phenobarbital; *CBZ*, carbamazepine.
*Same person.

lepsy had an effect on neurologic development, even when special emphasis was put on minor dysfunctions. No effect was found on behavioral development. This is in contrast with reports of Steinhausen et al.[37] and Hättig and Steinhausen[12] who indicated a slight increase of behavioral problems after fetal exposure to antiepileptic drugs. Skull circumference was the only clear-cut drug effect that we could establish; there was a negative association with phenobarbital exposure and, surprisingly, a positive one with carbamazepine exposure. This is in disagreement with Hilesma et al.[7] who found a more pronounced reduction of head size in carbamazepine-exposed children than in phenobarbital-exposed offspring. Deblay et al.[8] found a high rate of small head size in newborns exposed to a variety of antiepileptic drugs, mostly phenobarbital combined with other drugs, during fetal life and reported that the small head circumference was associated with impaired psychomotor development.

In our study the association between drug exposure and cognitive problems was weak, but to the disadvantage of phenobarbital. Our data indicate a counteracting effect of carbamazepine on the negative influence of phenobarbital. Possibly carbamazepine lowered phenobarbital serum levels inasmuch as it is known to induce hepatic enzyme activity. The possible difference in teratogenicity of phenobarbital and carbamazepine is in accordance with results from animal research that indicate phenobarbital should be regarded as a more potent teratogen than carbamazepine.[6]

Animal studies report growth retardation, microcephaly, and long-term effects on learning and behavior as a consequence of antenatal phenobarbital exposure.[38-41] It is possible that by matching our control group for birth weight we partially eliminated a negative influence of phenobarbital. Still mean birth weight in the phenobarbital group was lower than that in the other three study groups, and the difference between the phenobarbital and carbamazepine groups was significantly lower ($p = 0.005$; Table I). The study was not prospectively designed with random assignment of treatment with antiepileptic drugs. Explorative studies such as the present one rarely are. Drug type and dosages were prescribed during prenatal care, guided by the clinical condition. Throughout pregnancy, type and dosages remained constant. Of course the no-medication group comprised patients with a milder type of epilepsy, but in all cases the diagnosis was unequivocal. We did not find any effect of the number of antiepileptic drugs (zero, one, or two) on any of our outcome measures.

Our findings are the more remarkable inasmuch as the dosages of antiepileptic drugs were low, the pregnant women's epilepsy was well controlled, and con-

founders could be eliminated by careful matching. Phenobarbital is a potent neurotropic drug. It is known to produce a withdrawal syndrome in the newborn, and little is known about the late sequelae of withdrawal. When given to infants and preschool children phenobarbital may cause behavioral disturbances such as irritability, hyperactivity, and attention deficit.[42] It is conceivable that phenobarbital has also a neurotropic effect on the developing fetal brain, possibly with long-term consequences.

The offspring of epileptic mothers has a slightly increased risk of developmental anomalies. Part of this risk can be attributed to factors linked with the mother's disorder.

Evidence is gradually accumulating that the use of particular anticonvulsants during pregnancy is not as safe as is usually presumed for an epileptic mother's offspring. The present study adds evidence for a teratogenic effect of phenobarbital on skull circumference and possibly on cognitive development. Because both effects and groups are comparatively small, the findings urge for replication in a multicentered design to acquire a substantial number of cases.

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Multinuclear spermatozoa associated with polyploidy

Firuza R. Parikh, MD,[a] Margaret J. Iannielo, BS,[b] Michael Kashgarian, MD,[b] Alan H. DeCherney, MD[a] and Gad Lavy, MD[a]

New Haven, Connecticut

We are reporting on a possible association between multinucleated spermatozoa and polyploidy after in vitro fertilization. The semen from the male partner of a couple with 40% polyploidy on three in vitro fertilization attempts was examined by light and transmission electron microscopy. In that sample 10% to 12% of spermatozoa demonstrated conjoined or double heads. The significance of these findings is discussed. (Am J Obstet Gynecol 1991;164:128-9.)

Key words: Multinuclear spermatozoa, in vitro fertilization, polyploidy

Polyploidy occurs in 5% to 10% of human ova that are penetrated in vitro. Polyploidy can arise from polyspermy, which is defined as penetration of an oocyte by more than one spermatozoon, or it may result from the fusion of a diploid gamete with a haploid gamete. Diploidy arises from failure of the first or second meiotic division of gametes. Banding techniques have suggested that chromosomal heteromorphism is a possible mechanism of triploidy. A high rate of polyploidy was observed in several in vitro fertilization cycles in the same individual. This phenomenon appeared to be caused by insemination with spermatozoa from a sample that contained approximately 12% multinucleated forms.

Case report

Mr. and Mrs. R.M. were enrolled in the in vitro fertilization program at the Yale–New Haven Hospital for treatment of unexplained infertility. The husband was 43 years old, and the wife was 40 and nulliparous. Both were in otherwise good health. Semen analysis before enrollment showed a concentration of 59 million per milliliter with 15% abnormal forms.

Established protocols were used during in vitro fertilization. Human menopausal gonadotropin was used for ovarian stimulation, followed by administration of human chorionic gonadotropin in the first two cycles; leuprolide acetate; human menopausal gonadotropin and human chorionic gonadotropin were combined for the third. Thirteen ova were obtained by transvaginal aspiration, three each in the first two cycles and seven in the third cycle. They were staged according to the degree of corona dispersion and cumulus expansion. One oocyte was graded as postmature whereas the others were graded as mature. The semen specimen used in the three in vitro fertilization attempts revealed normal concentration and motility parameters. However, morphologic examination revealed a persistent abnormality. Approximately 10% to 12% of the spermatozoa displayed conjoined or double heads on light microscopy. The specimen obtained in the last cycle showed that 36% of the spermatozoa were pyriform or tapering. Each oocyte was inseminated with 0.5 million sperm prepared by the "swim-up" technique. At 16 or 18 hours after insemination the ova were examined for evidence of penetration and the number of pronuclei were noted. In 4 of the 10 eggs that were fertilized, more than two pronuclei were seen. This number is substantially higher than the incidence of polyploidy in

From the Departments of Obstetrics and Gynecology[a] and Pathology,[b] Yale University School of Medicine.
Received for publication October 6, 1989; revised May 2, 1990; accepted July 25, 1990.
Reprint requests: Gad Lavy, MD, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510.
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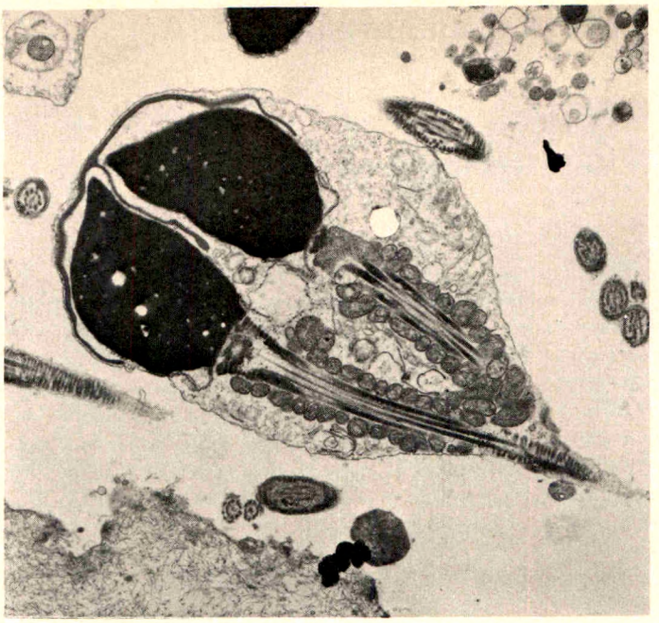


Fig. 1. Electron micrograph of a single-headed, binucleate spermatozoon.

our program (4.7%). None of the ova with polypronuclear development cleaved.

To further evaluate the cause of the high incidence of polyploidy in this couple and because ova appeared normal, the leftover semen specimen from the third in vitro fertilization attempt was examined by light and transmission electron microscopy. On light microscopy conjoined or double heads were found in 10% to 12% of the spermatozoa. Transmission electron microscopy revealed multinucleate heads in 12% of the spermatozoa. Fig. 1 shows a single-headed spermatozoon, but the plasma membrane surrounds two or more clearly individualized nuclei, which share a single acrosome that insinuates the interstices between them. The tail is double, at least in the initial region.

Comment

Utilizing banding techniques, Jacobs et al.[1] showed that 66.4% of 26 triploid spontaneous abortuses were due to dispermy, 23.6% were caused by fertilization of a haploid oocyte by a diploid spermatozoon, and 10% resulted from diploid ova that resulted from failure of the first meiotic division. Diploid spermatozoa are probably the result of failure of the spermatid intercellular bridges to separate. This abnormality is frequently seen after irradiation, and it is sometimes observed in hyperprolactinemia in conjunction with a pituitary tumor. The high incidence of 40% polyploidy together with the finding of 12% multinucleate spermatozoa suggests the latter as a possible cause. Although penetration occurred and resulted in polyploidy in this case, none of the preembryos cleaved, in comparison with the other preembryos that had normal penetration and cleavage.

In summary, electron microscopy was used to document an unusual sperm defect. The presence of multinucleate spermatozoa was associated with and may be related to recurrent polyploidy. In our patient it also helped us to establish that the spermatozoa were not conjoined, which is a rather frequent occurrence, but were multinucleate, which is a rare defect.[2]

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Therapy of persistent human papillomavirus disease with two different interferon species

Stanley A. Gall, MD, Lisa Constantine, RN, and Doris Koukol, RN
Louisville, Kentucky

Consensus interferon and interferon alfa-2a were used to treat patients with persistent human papillomavirus disease. Thirty-one patients were treated with either consensus interferon or a placebo (25 received active drug and 6 received placebo), and 24 patients were treated with interferon alfa-2a. Of the 25 patients who received consensus interferon, 19 (76%) showed either complete or partial clearing of condyloma 10 and 16 weeks after initiation of therapy; in 22 of the 24 patients (91%) who received interferon alfa-2a, clearing was demonstrated after a similar time period. Thirty of 34 patients who had no response or a partial response requested additional therapy, and 20 of these patients treated with adjunctive therapy had a complete response. The results of this study support the efficacy of both interferons in the treatment of overt condyloma acuminatum and suggest that interferon enhances subsequent adjunctive therapy. (Am J Obstet Gynecol 1991;164:130-4.)

Key words: Interferon, condyloma acuminatum, interferon alfa-2a, consensus interferon, human papillomavirus

The clinical management of human papillomavirus (HPV) infection of the female genital tract is controversial and nonstandardized because HPV infection is widespread (20% of women are estimated to harbor HPV) and because HPV deoxyribonucleic acid (DNA) is found in most squamous cancers of the female genital tract.[1-5] These two facts intensify the clinical problems that result from the lack of knowledge about the biologic features and natural history of HPV infections. Clinicians are only now realizing that HPV infection usually involves the entire lower female genital tract and that management of subclinical HPV infection is even more controversial than that for overt condyloma.

There is a lack of consensus about the appropriate management of HPV infections in the female genital tract. A recent treatment approach involves the use of interferon, both as primary therapy and as adjunctive therapy. Alpha, beta, and gamma interferons have been reported successful in the therapy of HPV infections.[6-10] The majority of the studies have reported the use of alpha interferon, but the details of dose, dosage schedules, frequency, and route of administration vary considerably.[11] The variety of regimens increases the difficulty of assessing the efficacy of interferon therapy in various stages of HPV disease.

This study was undertaken to assess the effectiveness of two types of interferon in the therapy of resistant and/or persistent HPV infection of the female genital tract. The interferons chosen were consensus interferon (r-Met-Hu IFN-Con) and interferon alfa-2a. Consensus interferon is a product of a synthetic gene for r-Met IFN-Con, which was derived by selecting the most frequently observed amino acid residues at each position of the known members of the interferon family of proteins. Interferon alfa-2a was chosen because it represents a single alpha interferon.[1]

From the Department of Obstetrics and Gynecology, University of Louisville School of Medicine.
Received for publication October 17, 1989; revised April 20, 1990; accepted August 7, 1990.
Reprint requests: Stanley A. Gall, MD, Department of Obstetrics and Gynecology, University of Louisville School of Medicine, Louisville, KY 40292.
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Material and methods

The prospective randomized trial was conducted from 1986 to 1989. Both female patients and one male patient were included in the study only if they had persistent and/or resistant disease. Persistent disease was defined as the presence of HPV manifested as condyloma acuminata for >6 months and 10 or more previous therapies. Therapies could have been podophyllin, trichloroacetic acid or 5-fluorouracil application, surgical removal, fulguration, cryotherapy, or laser therapy. Histologic confirmation of condyloma acuminatum was required. An informed consent form approved by the Institutional Review Committee was signed by each patient who participated in the study. Patients excluded from study included pregnant women or women who were breast-feeding; patients with additional dermatologic disease in the area of condylomas, which could preclude clinical evaluation of the condylomas; patients with granulocyte count $<2000/mm^3$ or platelet count $<100,000/mm^3$; patients with renal dysfunction as measured by a serum creatinine level >1.4 mg/dl; patients with serum glutamic oxaloacetic transaminase >120 U; patients with total

bilirubin level >2.0 mg/dl; patients with symptomatic heart disease, chronic obstructive pulmonary disease, liver disease, malignancy, psychiatric disorder necessitating medication, or any neurologic disorder; patients who were receiving drug therapy for HPV during the prior 14 days; patients with human immunodeficiency virus seropositivity; and patients with evidence of significant immunosuppression as determined by clinical evidence of opportunistic infection or treatment with immunosuppressive drugs.

Patients were prospectively randomized to receive one of five different dosing schedules of consensus interferon or a schedule of interferon alfa-2a. After inclusion in the study, the patients underwent general physical and neurologic examination. The pelvic examination included visual inspection of the perianal area, vulva, vagina, and cervix. Papanicolaou's test and biopsies of genital tract condylomas were done. The vulvar condyloma were documented by recording the bidimensional measurements on a divided grid of the external genitalia. Photographs of the vulva and perineum were made initially and at each subsequent evaluation. Patients were examined at entry and at 2, 4, 6, 10, 14, and 22 weeks during the study. Laboratory evaluations of complete blood count, platelets, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, blood urea nitrogen, and creatinine, as well as lesion measurements and photography, were done at every office visit. HPV DNA typing was not done in this study. Papanicolaou's test was not used to assess response to the interferons.

Consensus interferon was self-administered by intramuscular injection by a protocol that used five different dosing schedules for a 6-week period. These schedules were: schedule 1—high dose, three times per week; schedule 2—low dose, three times per week; schedule 3—high dose, two times per week, and one placebo injection per week; schedule 4—low dose, two times per week, and one placebo injection per week; schedule 5—placebo injection, three times per week. (High dose = 5×10^6 units, or 5 MU; low dose = 2.5×10^6 units, or 2.5 MU; MU = 1×10^6 units.)

The interferon alfa-2a was self-administered intramuscularly at a dose of 3 MU three times per week for 6 weeks, with possible extension of an additional 4 weeks of treatment.

Additional therapy-protocol management decisions were based on global disease assessments as follows: Complete response = total disappearance or clearance of overt warts; partial response = decrease in total wart size by ≥50%. Any change of <50% decrease in wart size was considered nonresponse. Patients were followed up as previously described, and frequently other clinical modalities were used in the nonresponse and partial response categories after the 14-week or 16-week visit.

Symptomatic side effects to interferon treatment which included fever, chills, fatigue, malaise, nausea and vomiting, and headaches, were recorded daily by patients. Clinical laboratory values and side effects were evaluated during each office visit.

Results

Fifty-five patients (54 women and one man) with resistant and persistent HPV infection as manifested by condyloma acuminata were included in this study of the efficacy of interferon therapy. Twenty-four patients received interferon alfa-2a and 31 patients participated in the consensus interferon protocol in which six patients received placebo only. Study participants consisted of 49 white patients, 5 black patients, and 1 oriental patient. Participants ranged from 6 to 50 years (mean, 27.3 years in the interferon alfa-2a group and 28.4 years in the consensus interferon group). One child, age 6, was treated with interferon alfa-2a. All other participants were 18 years of age or older. Baseline characteristics included a mean time of 32.6 months (range, 6 to 240 months) with disease after diagnosis for patients in the interferon alfa-2a group, as compared with a mean of 14.2 months (range, 6 to 24 months) for patients in the consensus interferon group. All female patients included in the study had vulvar lesions that ranged from several small overt condylomas to massive confluent warts. In addition to the vulvar disease found in the interferon alfa-2a group, 10 of the 24 patients (42%) had vaginal condylomas and 6 of the 24 patients (25%) had cervical condylomas. In the consensus interferon group, in addition to vulvar warts, 17 of the 30 patients (57%) had vaginal condylomas and 4 of 30 patients (13%) had cervical condylomas. Also two patients in the interferon alfa-2a group had grade 3 vulvar intraepithelial neoplasia lesions in addition to extensive condyloma. The one male patient had both perianal and penile condyloma.

Thirty-one patients were included in the consensus interferon group, but six patients received placebo only and 25 patients received active drug. The evaluation at 10 weeks after the initiation of the interferon showed that 6 of 25 patients (24%) experienced clearance of disease, 14 of 25 (56%) were partial responders, and 5 of 25 (20%) did not benefit from interferon therapy. When this same group was evaluated at 14 to 16 weeks after the initiation of therapy, 9 of 25 patients (36%) were free of overt disease, 11 of 25 (44%) were partial responders, and 5 of 25 (20%) continued to be nonresponders. The response to individual dosing schedules is shown in Table I. Obviously the number of patients per individual schedule is too small to be significant, but no patient in the placebo group responded and the response in the remaining groups seemed to be similar. Patients received total interferon doses according to schedules as follows: schedule 1, 90 MU;

Table I. Response to consensus interferon by different dosing schedules: Evaluation at follow-up

	10 wk			*14-16 wk*		
	CR	*PR*	*NR*	*CR*	*PR*	*NR*
Schedule 1	1	5	0	2	4	0
Schedule 2	1	4	1	3	2	1
Schedule 3	2	2	2	2	2	2
Schedule 4	2	3	2	2	3	2
Schedule 5	0	0	6	0	0	6

CR, Complete response; *PR*, partial response; *NR*, nonresponse.

Schedule 1: 5 MU t.i.w. × 6 weeks. Schedule 2: 2.5 MU t.i.w. × 6 weeks. Schedule 3: 5 MU b.i.w. and 1 placebo × 6 weeks. Schedule 4: 2.5 MU b.i.w. and 1 placebo × 6 weeks. Schedule 5: placebo t.i.w. × 6 weeks.

schedule 2, 45 MU; schedule 3, 60 MU; schedule 4, 30 MU. The average total dose of interferon was 55.2 MU.

Twenty-four patients were included in the interferon alfa-2a group. Six of 24 patients (25%) in the interferon alfa-2a group experienced clearing of condyloma at the 10-week evaluation, 16 of 24 (66%) were partial responders, and 2 of 24 (8%) experienced no benefit from interferon treatment. Evaluation of this group at 14 weeks showed that 12 of 24 (50%) were complete responders, 10 of 24 (42%) were partial responders, and 2 of 24 (8%) were still nonresponders to interferon therapy. The average total dose of interferon alfa-2a was 88 MU (range of 54 to 216 MU). Seventeen of the 24 patients received interferon alfa-2a after the initial 6-week treatment period. The duration of extended treatment was 2 weeks in 11 patients, 4 weeks in three patients, and 6 weeks in three patients. Of the 11 patients who received an additional 2 weeks of therapy, four patients became complete responders, five patients remained partial responders, and two patients remained nonresponders. Of the three patients who received an additional 4 weeks of interferon, two became complete responders and one remained a partial responder; of the three patients who received an additional 6 weeks of interferon, two remained partial responders and one became a complete responder.

Patients who had completed study protocols were offered adjunctive therapy. The results are shown in Table II. At the conclusion of the study protocol, 21 of 49 patients (43%) who had received interferons experienced complete clearing of lesions, whereas 6 of 6 (100%) who had received placebo showed no clinical benefit. Thirty of 34 patients who had a partial response or no response requested additional therapy. Fifteen patients (50%) received additional interferon, and 5 of 30 patients (17%) were treated with cryotherapy, laser therapy, or surgical excision. Six patients (20%) received either 5-fluorouracil or 85% trichloroacetic acid; four patients (13%) received combination therapy with trichloroacetic acid plus laser, 5-fluorouracil, or cryotherapy or 5-fluorouracil plus trichloroacetic acid. When the efficacy of all adjunctive therapies is reviewed, 20 of the 30 patients (67%) treated were judged to be complete responders 3 months after initiation of adjunctive therapy, eight of the 30 patients (27%) were considered partial responders, and six (20%) patients were lost to follow-up. Four of seven patients treated with interferon who had been nonresponders showed complete response, and three of seven showed a partial response. The adjunctive therapy was successful in the group of six patients who had originally received the placebo. Four of these six patients experienced a complete response, one patient had a partial response, and one patient was lost to follow-up.

Biologic side effects of treatment included fever, chills, fatigue, and malaise in almost all patients at the initiation of therapy. Fever usually occurred only with the first several injections. The most pervasive symptom was fatigue, which continued to some extent in almost every patient until interferon therapy was discontinued. Both consensus interferon and interferon alfa-2a caused similar symptoms, the extent of which was dose-related. Unusual symptoms included weight loss of >10% in one patient and moderate hair loss in two patients. No episodes of leukopenia (≤3000 white blood cells/mm^3) or thrombocytopenia (platelets ≤100,000/mm^3) occurred in any of the patients who had received interferon.

No patient was excluded from the study on the basis of seropositivity. No experience has been obtained in using interferon therapy for treating condyloma acuminatum in patients who are seropositive for human immunodeficiency virus.

Comment

The objective of this research was to determine the efficacy of two different types of alpha interferon. Consensus interferon is a designer molecule produced by *Escherichia coli* through the expression of a synthetic gene that is carried on a plasmid vector. This interferon is different from other alpha interferons in that it consists of an amino acid sequence that reflects the most frequently occurring amino acid residues at each position of the 13 known members of the alpha interferon

Table II. Subsequent therapy after completion of interferon study protocols

Original interferon	n	Final follow-up evaluation at 14-16 wk			Subsequent therapy					3 mo follow-up of subsequent therapy			
		CR	PR	NR	IFN	5-FU or IFN	Cryosurgery or laser	5-FU or TCA	Other*	CR	PR	NR	LTFU
Consensus	25	9	11	5	5	4	1	3	3	11	2	0	3
Placebo	6	0	0	6	4	1	0	0	1	4	1	0	1
Interferon alfa-2a	24	12	10	2	0	1	4	3	4†	5	5	0	2

CR, Complete response; *PR*, partial response; *NR*, nonresponse; *IFN*, interferon; *5-FU*, 5-fluorouracil; *TCA*, trichloroacetic acid; *LTFU*, lost to follow-up.

*One patient with 5-fluorouracil + laser, one with 5-fluorouracil + trichloroacetic acid, one with trichloroacetic acid + cryotherapy, and one with trichloroacetic acid + laser.

†Lost to follow-up, 2; no therapy, 2.

family of proteins. The 166 amino acid consensus protein has a molecular weight of 19500 d, as do natural leukocyte interferons. The natural subtype interferon F is the alpha interferon that is most closely related to r-Met-Hu IFN-Con_1 (consensus interferon). There are only 10 positions at which consensus interferon differs from interferon F. Comparison of interferon B and consensus interferon shows 57 common amino acid positions. Thus consensus interferon is more similar to beta interferon than to a natural alpha interferon subtype with >30% homology.

One of the first interferons cloned was interferon alfa-2a. This naturally occurring interferon is now mass-produced by recombinant technology that uses *E. coli* to express a gene carried on a plasmid vector. Interferon alfa-2a therapy has been extensively used in clinical trials.

Interferon acts like other cytokines; when it is released into circulation, it interacts with a specific receptor.[12] Alpha and beta interferons appear to have a glycoprotein receptor.

The antiviral, anticellular, and immunomodulatory activities of interferon result from the internalized receptors that send signals to the appropriate cellular genes. Interferon inhibits cell growth in many cell types. Although there is marked cell-to-cell variation in sensitivity,[13] there is also a bimodal response in differentiation of cells, depending on the dose of interferon. With low-dose interferon, there may be an enhancement of differentiation, whereas there may be an inhibition of differentiation with high-dose interferon in various cell systems.[14]

The effects of interferons on the immune system are varied and are both cell-specific and interferon-specific. Interferons appear to inhibit the proliferation of cells, to enhance natural killer cell activity, and to enhance T-cell cytotoxicity and macrophage activation.[15-17] These considerations are important because minor depressions of the immune system appear to allow HPV either to become activated from latency or to remain clinically active. Patients with immunosuppressive diseases or those who receive exogenous corticosteroids may have activation of HPV disease. The use of interferon treatment in these patients is rarely successful.

The overall clinical response indicates that the two types of interferon treatment examined in this study have similar efficacy in the therapy of HPV disease. However, patients in the interferon alfa-2a group seemed to be a different population, disease had been present in these patients for a mean of 32.6 months before initiation of therapy, as compared with 14.2 months in patients treated with consensus interferon. It has been recognized that the longer the disease is clinically present, the less the likelihood for spontaneous remission. However, it is unknown whether disease present for 32.6 months is biologically different from disease present for 14.4 months.

Two patients in the interferon alfa-2a group had extensive grade 3 vulvar intraepithelial neoplasia in addition to extensive vulvar condyloma acuminatum. The administration of interferon alfa-2a caused a >50% decrease in the volume of condyloma but did not affect the grade 3 vulvar intraepithelial neoplasia. These two patients were subsequently treated with surgical removal of the neoplasia.

Patients in the interferon alfa-2a group received a mean of 88 MU per patient, as compared with 55.2 MU per patient in the consensus interferon group. The interferon alfa-2a protocol allowed for a continuation of the interferon alfa-2a in partial responders if it was thought to be clinically indicated. However, the results of this protocol suggest that the additional length of administration in the nonresponsive patient did not improve the overall clinical efficacy.

An important contribution of this study is to confirm

the value of adjunctive therapy in the treatment of HPV disease. Thirty of 34 patients eligible for additional adjunctive therapy chose to be treated; 20 of these 30 (67%) experienced complete clearing of condyloma. Therefore 41 of 55 patients (75%) included in this study were cleared of overt disease, whereas 8 patients were partial responders and 6 patients were lost to follow-up evaluation. The adjunctive therapy consisted of a variety of modalities; the largest number of patients received additional interferon therapy. It has been shown previously that physical ablation after interferon therapy increased clinical clearing; this suggests that further study is necessary.

The knowledge of the natural biologic characteristics of HPV infection is not well understood. The most effective therapy for the management of HPV is yet to be verified. However, data from this study support those of previous studies, which indicate that interferon may be used either as a primary treatment or as adjunctive therapy, either in conjunction with physical ablation or in combination with trichloroacetic acid or 5-fluorouracil. In addition, it is clear that any depression of cell-mediated immunity may allow growth of HPV or may make its control more difficult. Therefore patients with HPV disease must be instructed to stop smoking, since smoking causes mild immunodepression. More than 85% of the women with HPV disease in this study were smokers as compared with approximately 35% of women with HPV infection in the general population.

This study supports the concept that both interferons (consensus interferon and interferon alfa-2a) are effective in the therapy of overt condyloma acuminatum. Additional trials should be supported to determine the appropriate dosing for sequential therapy with laser ablation, cryotherapy, or trichloroacetic acid application. There is no evidence that the number of patients infected with HPV is decreasing, and newer approaches to management must be developed.

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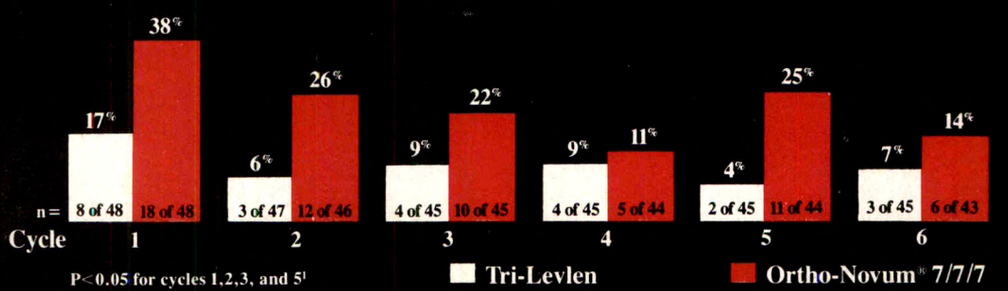
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Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions. 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000); the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert. 60611-1 5/86

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Cross-sectional analysis of triplet birth weight

J. Stephen Jones, MD,[a] Roger B. Newman, MD,[a] and M. Clinton Miller, PhD[b]
Charleston, South Carolina

Parameters of fetal growth in triplet gestations are poorly studied and controversial. A cross-sectional analysis of triplet birth weight was performed to elucidate fetal growth patterns. Birth weight and gestational age data were analyzed on 580 infants in 196 triplet sets (eight stillborn infants excluded) between 1985 and 1988. Ovulation induction was used in approximately one half the gestations and early obstetric and ultrasonographic dating was available in all pregnancies. The mean triplet set and individual triplet weights versus gestational age were calculated with distinctly linear growth displayed between 22 to 38 weeks' gestation. Mean intratriplet differences at all gestational ages were determined. A comparison of singleton and triplet growth curves was constructed to show the distinct growth characteristics of triplets. (AM J OBSTET GYNECOL 1991;164:135-40.)

Key words: Triplets, fetal growth, intratriplet difference

Cross-sectional analysis of live-born birth weight data has historically been the basis for the establishment of intrauterine growth curves for singleton and twin pregnancies.[1-3] With the use of such growth curves it is possible to study factors that influence perinatal growth, and with the use of ultrasonography to follow intrauterine growth prospectively.

Although multiple gestations account for only 1% of all live-born births in the United States, they constitute 10% to 12% of perinatal deaths and 17% of intrauterine growth retardation.[4] Triplet gestations, as a subset of multiple gestations, occur spontaneously in approximately one in 10,000 live-born pregnancies.[5] Perinatal mortality rates ranging from 15% to 31% for triplet gestations have been reported.[6] In addition, the incidence of triplet birth is increasing because of the use of ovulation induction and in vitro fertilization.[7]

Data with regard to intrauterine growth parameters for triplets remain scarce. It is necessary to develop accurate growth curves for triplet gestations to aid our understanding of triplet growth, to assist in the diagnosis of intrauterine growth retardation, and it is hoped, to improve perinatal survival.

Methods

Anthropometric data from 196 triplet pregnancies delivered between 1985 and 1988 were analyzed. A description of the triplet pregnancies and details concerning their collection methods were previously published.[7] Data were collected with the assistance of ambulatory perinatal nurses employed by the Tokos Medical Corporation. The perinatal nurses were in daily contact with these women for the purpose of antepartum surveillance and preterm birth prevention. Individual patient management was at the discretion of a large number of attending obstetricians in both private and academic practices.

Data from 580 infants from these 196 triplet sets were analyzed; eight stillborn infants were excluded from the data set. Gestational dating was determined on the basis of the date of in vitro fertilization in 13 pregnancies and by the date of ovulation in 90 induced triplet gestations. In 93 spontaneous triplet gestations the estimated date of conception and gestational age were established on the basis of early obstetric and ultrasonographic criteria. Specific zygosity determinations were not available.

Birth weight (grams) was recorded for each live-born member of the triplet set as well as each triplet set. The gestational age and birth weight for individual triplets and triplet sets are reported as mean ± SD ($\bar{X} \pm SD$). Individual triplet birth weight descriptive statistics were performed independently of triplet sets because of the exclusion of the eight stillborn infants.

Growth curves were constructed by grouping infants according to gestational age. Gestational age at delivery was assigned by rounding to the nearest whole week (i.e., 32 3/7 weeks reported as 32 weeks, 32 4/7 weeks reported as 33 weeks). Statistical analysis of growth curves for both individual and triplet sets was performed with simple regression analysis. The first and second SDs were constructed by simple regression analysis with lines constructed from statistically derived SDs. The correlation coefficient was used to evaluate the accuracy of the presented growth curves.

Intratriplet difference was calculated as the differ-

From the Departments of Obstetrics and Gynecology[a] and Biometry,[b] Medical University of South Carolina.
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Reprint requests: J. Stephen Jones, MD, Department of Obstetrics and Gynecology, SGHSO, Keesler Technical Training Center Medical Center, Keesler Air Force Base, MS 39534-5300.
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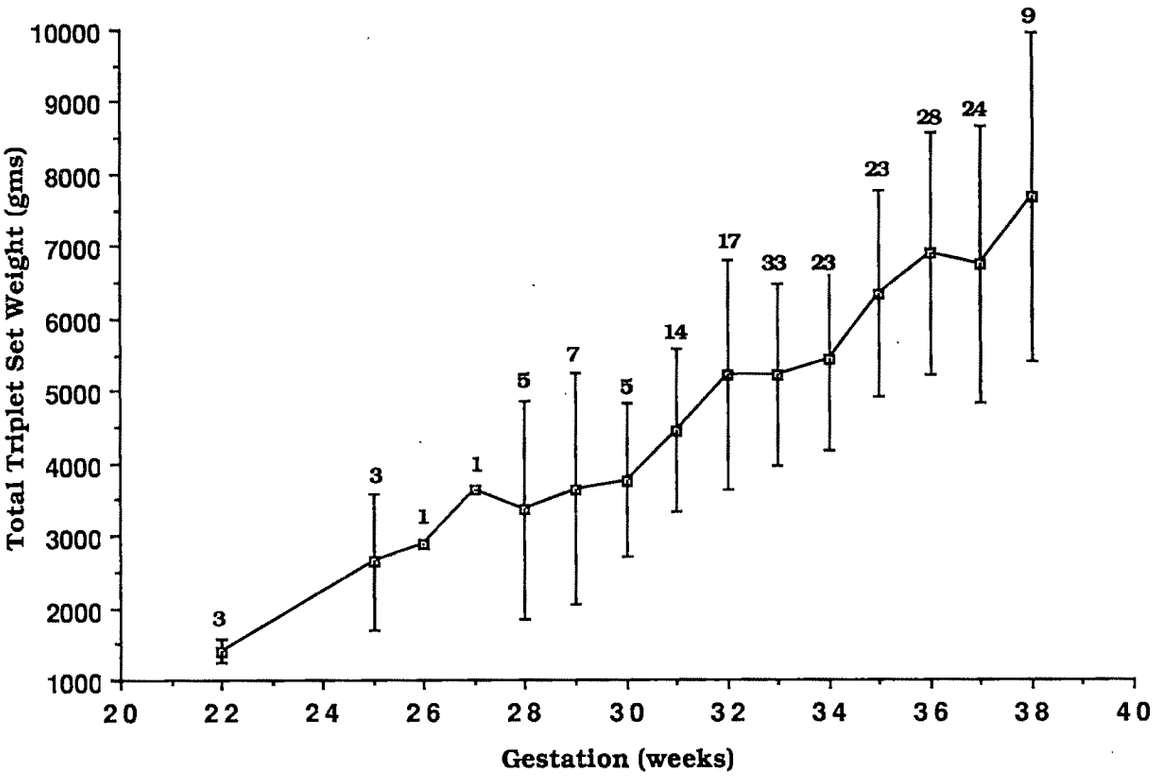


Fig. 1. Mean triplet set birth weights (N = 196) in grams and 95% confidence limits by gestational age at delivery. Number of triplet sets delivered at each week gestation is listed above confidence band.

Table I. Triplet birth weight data

Gestational age (wk)	*Triplets (No.)*	*Individual triplet weight (gm)**	*Triplet set weight (gm)**
22	9	468 ± 27	1403 ± 87
25	9	877 ± 148	2631 ± 473
26	3	964 ± 74	2891 ± —
27	3	1210 ± 32	3629 ± —
28	15	1118 ± 249	3352 ± 754
29	19	1105 ± 307	3634 ± 799
30	15	1254 ± 245	3762 ± 531
31	40	1496 ± 270	4452 ± 563
32	50	1725 ± 326	5203 ± 792
33	98	1742 ± 286	5218 ± 626
34	68	1807 ± 293	5420 ± 618
35	69	2110 ± 380	6330 ± 709
36	83	2288 ± 406	6894 ± 841
37	72	2250 ± 400	6751 ± 957
38	27	2557 ± 434	7670 ± 1135

*Mean ± SD.

ence between the largest and smallest triplet of each set. This difference is referred to as the maximum intratriplet difference. The percent maximum discordance was obtained by dividing the maximum intratriplet difference by the largest triplet of the set multiplied by 100. The individual triplet growth curve was compared with an established singleton curve.[2]

Results

The mean gestational age at delivery for this triplet series is 33.6 ± 3.1 weeks. The mean birth weight for each individual triplet is 1864 ± 543 gm and the mean birth weight for each triplet set is 5593 ± 1488 gm. Table I provides the number of triplets delivered at each gestational week along with the corresponding individual and triplet set birth weights (mean ± SD).

Fig. 1 shows the mean triplet set birth weight plotted against the gestational age at delivery. Triplet set growth is best described by the linear regression equation: W = −7737.1 + 402.56 (WG), where W = weight (grams) and WG = weeks' gestation at delivery. The correlation coefficient (R^2) between triplet set weight and gestational age is 0.956.

Fig. 2 shows the mean individual triplet birth weight plotted against the gestational age at delivery. A simple linear regression model best describes individual triplet growth (R^2 = 0.972) between gestational weeks 22 to 38. The equation for the line is as follows: W = −2311.3 + 124.73 (WG). On the basis of the linear growth model, individual triplets are predicted to gain approximately 125 gm per week during this linear phase of growth. Fig. 3 presents a depiction of the

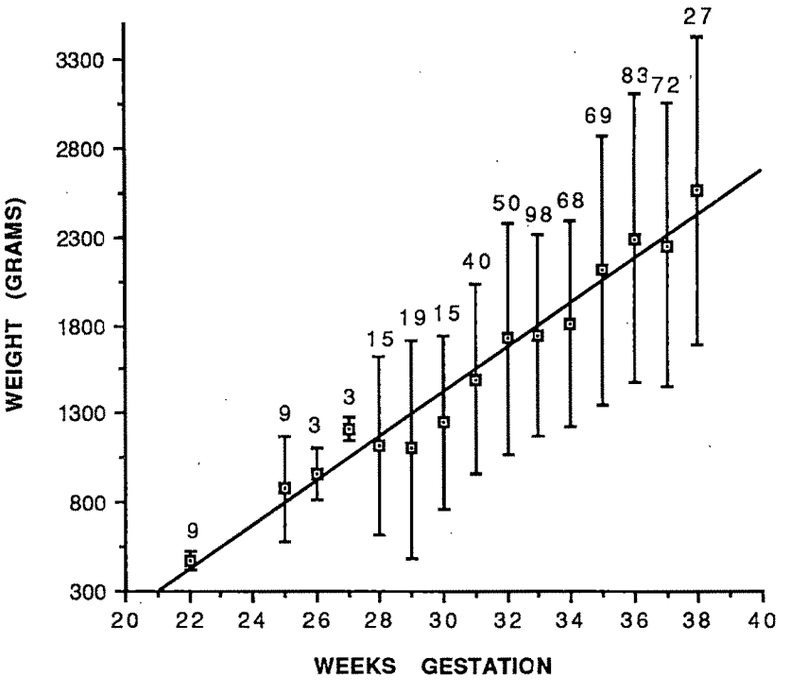


Fig. 2. Mean individual triplet birth weights (N = 580) in grams and 95% confidence limits by gestational age at delivery. Number of individual triplets delivered at each gestational week is listed above confidence bands. Regression line of best fit (R = 0.98) is superimposed on data.

individual triplet growth curve along with ± 1 and 2 smoothed SD linear regression lines.

For all the triplet sets the mean maximum discordance is 20.0%. Table II provides the mean maximum intratriplet weight difference at each gestational week (mean ± SD). Also presented is the calculated percent maximum discordance at each gestational week. Fig. 4 displays the histogram distribution of percent maximum discordance.

Approximately 80% of the triplet pregnancies were in white women. The triplet pregnancies in nonwhite women were nonuniformly distributed throughout all gestational ages, thus prohibiting statistical comparisons of racial differences.

Comment

The only previous cross-sectional analysis of triplet birth weight of a comparable size was reported by McKeown and Record[8] in 1952. In their article, 249 triplet gestations with 747 newborns beyond 28 weeks' gestation were analyzed. Gestational age was determined entirely on the basis of the onset of the last menstrual period. With the use of regression analysis it was determined that the mean fetal weight was similar for singleton and multiple gestations through the first two trimesters of pregnancy. At 27 weeks' gestation in triplets and at 30 weeks' gestation in twins, McKeown and Record found that the rate of growth appeared to decline progressively compared with singleton gestations.

Our data, collected between 1985 and 1988, represent a cross-sectional analysis of 196 triplet gestations with 580 live-born infants. Early gestational dating of these pregnancies on the basis of a known date of ovulation in over one half of these pregnancies and by contemporary obstetric and ultrasonographic criteria in the remainder ensure the accuracy of the cross-sectional analysis of our data.

Regression analysis of triplet growth for both individual and triplet sets reveals that the linear growth model best describes triplet growth between 22 and 38 weeks' gestation. Growth curves of higher order polynomial expressions could not improve on the linear model. The correlation coefficients of 0.956 for triplet sets and 0.972 for individual triplet growth are highly significant and indicate an excellent linear relationship.

Naeye et al.[3] supported McKeown and Record's observation that the intrauterine growth of twins did not parallel singleton growth. They concluded from live-born twin birth weight data that twin growth is similar to that of singletons until 30 weeks' gestation at which time twin growth "decreases" with respect to singletons throughout the remainder of pregnancy. With the use

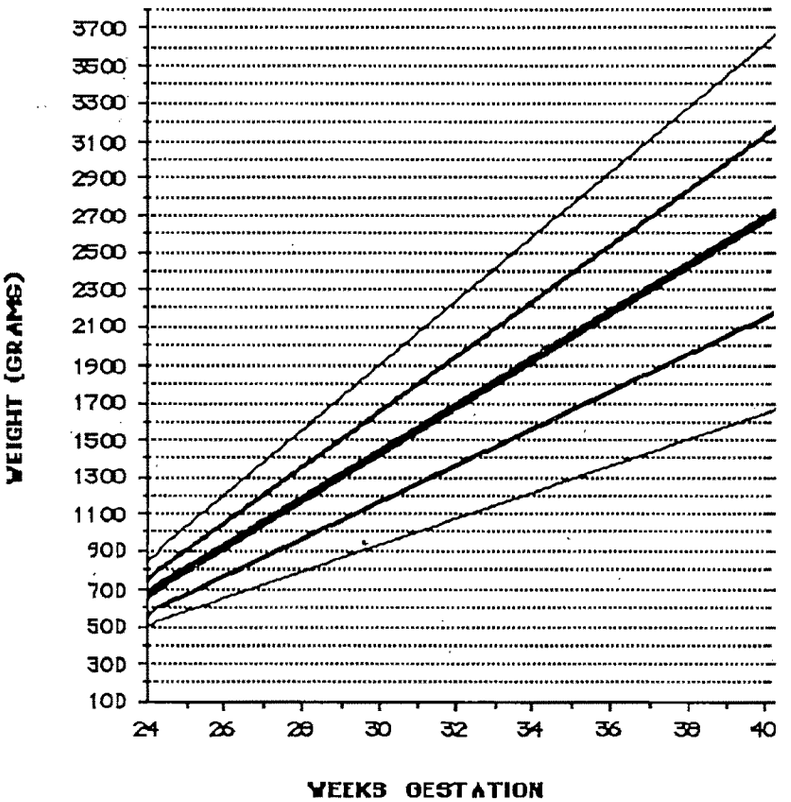


Fig. 3. Individual triplet growth curve. Mean and smoothed first and second SDs for birth weight according to gestational age are graphed.

Table II. Intratriplet set weight differences

Gestational age (wk)	*Triplet sets (No.)*	*Mean maximum ITD* (gm)*	*Discordance (%)†*
22	3	19 ± 27	3.9
25	3	118 ± 148	12.5
26	1	141 ± 74	13.8
27	1	56 ± 32	4.5
28	5	170 ± 249	15.2
29	7	58 ± 307	6.3
30	5	357 ± 245	27.5
31	14	378 ± 270	22
32	17	397 ± 326	21.5
33	33	372 ± 286	20.1
34	23	425 ± 293	20.3
35	23	564 ± 380	23.2
36	28	562 ± 406	23
37	24	480 ± 400	19.7
38	9	339 ± 434	15.1

**ITD*, Intratriplet difference, calculated as largest minus smallest triplet ± SD.

†Calculated as maximum intratriplet difference/largest triplet × 100.

of live-born birth weight data of twins Daw and Walker[9] also identified a "diminished rate of growth" for twins in the third trimester.

Our data on triplet growth when compared with previously reported singleton growth (Fig. 5) supports the concept that multiple and singleton gestations exhibit similar growth through 30 weeks. Beyond 30 weeks' gestation, the singleton fetus appears to accelerate in growth in a sigmoid manner. The triplet gestation lacks this acceleration in growth shown by singleton gestations. However, triplet growth remains linear throughout the third trimester rather than plateauing as previously suggested. The failure to identify an acceleration of growth in triplet pregnancies may be a result of suboptimal transfer of nutrients by the uteroplacental circulation in the third trimester as previously suggested for twin gestations.[10]

McKeown and Record previously speculated that individual growth in multiple gestations was not compromised until the total fetal weight exceeded 7 pounds (approximately 3200 gm). After 38 weeks in singleton pregnancies (corresponding to 3000 to 3200 gm), fetal growth begins to show a plateau effect. Triplets in our series continued to have linear growth well beyond a total intrauterine weight of 3200 gm that occurred at ≃ 28 weeks. The difference in growth also could be explained by contemporary antepartum management, including extensive bed rest and obstetric intervention for abnormal growth determined by ultrasonography.

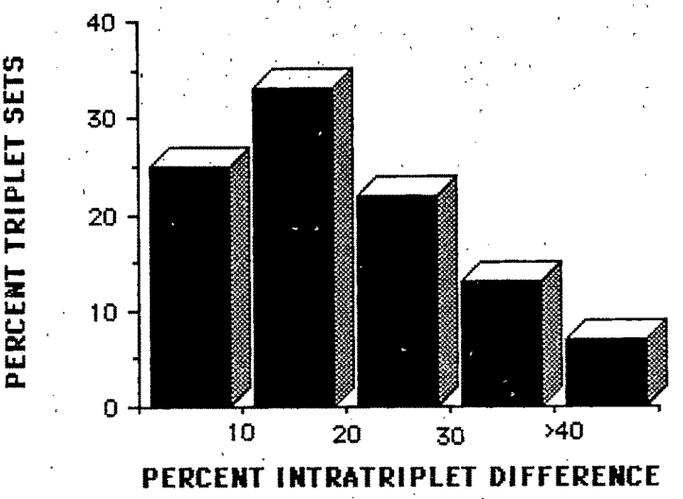


Fig. 4. Histogram of maximum triplet discordance. Vertical axis represents percentage of triplet sets displaying percentage of maximum triplet discordance, which is shown on *horizontal axis*. Maximum triplet discordance percentage defined as largest–smallest/largest × 100.

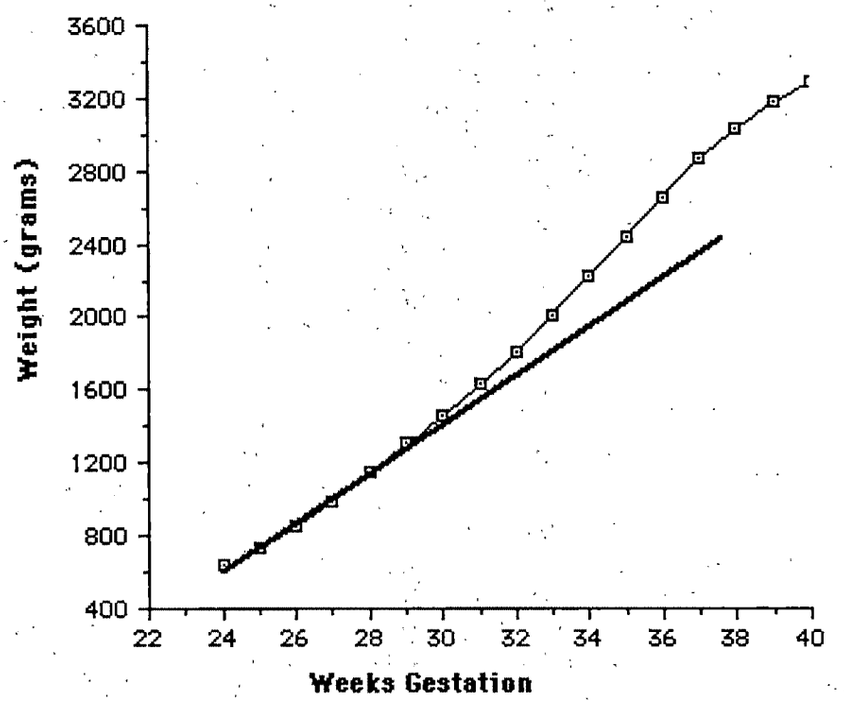


Fig. 5. Individual triplet growth curve *(solid black line)* compared with previously published singleton growth curve *(interrupted black line)*. The latter curve is from Brenner WE, Edelman DA, Hendricks CH. Am J Obstet Gynecol 1976;126:555-64.

Unfortunately, factors potentially affecting fetal weight gain including maternal smoking history, prenatal weight gain, and maternal stature were not available for statistical analysis.

Information derived from the analysis of intratriplet differences was very surprising. Discordance in twin growth is usually defined as a difference in a body weight exceeding 25%.[11] Mean intertwin weight differences at birth have been reported to be 10.3%.[12] Discordance can result from several factors including placental implantation differences, twin-to-twin transfusions, anomalies, and inherited genetic differences.

In this review 30.1% of the triplet gestations had an intratriplet discordance of at least 25% as determined

by the difference between the largest and smallest member of each triplet set. This compares with approximately 5% of twin pairs that display at least 25% discordance.[13] The histogram analysis of discordance suggests that approximately 7% of triplet sets displayed >40% discordance. We are unable to comment on the perinatal implications of such large degrees of discordance, but it appears that intratriplet growth differences are more pronounced than with twin gestations and the antepartum management implications may be different.

Singleton and twin growth curves have both been used to follow up triplet growth prospectively. Although ultrasonography is a useful instrument to predict fetal weight, the lack of accurate triplet growth curves limits our ability to interpret triplet growth. Regression analysis of mean triplet birth weight and gestational age allows the construction of a growth curve for triplets (Fig. 3).

Prospective ultrasonographic evaluation of twin gestations has been shown to be a sensitive method to detect abnormal growth and has resulted in a reduction of perinatal morbidity and mortality.[14] It is hoped that the establishment of a triplet growth curve with limits of variability defined by lines fitted to 1 and 2 SD lines at established weekly intervals will be helpful to prospectively follow triplet growth and reduce triplet morbidity and mortality.

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The effects of indomethacin and a β-sympathomimetic agent on the fetal ductus arteriosus during treatment of premature labor: A randomized double-blind study

Marianne Eronen, MD,[a] Erkki Pesonen, MD,[a] Tapio Kurki, MD,[b] Olavi Ylikorkala, MD,[b] and Mikko Hallman, MD[b, c]

Helsinki, Finland

To study the effect of maternal indomethacin or nylidrine hydrochloride treatment on the fetal ductus arteriosus and the pulmonary artery, 27 women with threatened preterm labor between 24 and 34 weeks' gestation were studied by echocardiography. Fourteen women were treated with indomethacin and 13 with nylidrin. Both systolic and diastolic velocities in the ductus increased after administration of indomethacin indicating constriction in nine fetuses, and exceeded the corresponding velocities in the fetuses of the nylidrin group ($p = 0.001$). However, there were no changes in pulmonary artery flow velocities ($p > 0.5$). In the indomethacin group, there was a significant linear positive relationship between the gestational age and the change in ductal flow velocity. Three of the nine patients with ductal constriction also had tricuspid regurgitation. These findings indicate that indomethacin, not nylidrin, causes transient constriction of the ductus arteriosus and the constrictive response increases with the gestational age. We recommend echocardiographic surveillance of fetal hemodynamics when prostaglandin synthesis inhibitors are used in the treatment of spontaneous preterm labor. (AM J OBSTET GYNECOL 1991;164:141-6.)

Key words: Ductal blood flow velocity, human fetus, indomethacin, premature labor

Spontaneous premature delivery is the main cause of prematurity, accounting for a major part of serious neonatal morbidity and mortality.[1] Tocolytic therapy with β-sympathomimetic agents has been of minor or no significance in arresting preterm labor.[2] In addition, these agents have potentially harmful cardiovascular effects on both the mother[3] and fetus.[4] Because prostaglandins have an essential role in initiation and progression of labor,[5] the use of inhibitors of synthesis could be a logical approach to stop or delay preterm labor.[6, 7] These drugs may be a double-edged sword; besides inhibiting the production of uterine prostaglandins they also inhibit the production of fetal prostanoids, such as prostaglandin E_2 and prostacyclin, which are needed to keep the ductus open. In experimental animals, indomethacin and other cyclooxygenase inhibitors constrict the fetal ductus arteriosus,[8] increasing pulmonary vascular resistance and causing persistent fetal circulation with neonatal death.[9, 10] There are few case reports on pulmonary hypertension in the newborn[11-13] after the administration of indomethacin. Prospective trials with indomethacin have shown evidence of postponing premature labor,[11] and there has been little or no evidence of harmful effects in the neonates.[15-17] A recent study[18] presented evidence that indomethacin administration significantly increased the blood flow velocity in ductus arteriosus suggesting ductal constriction in seven of 14 fetuses studied. This suggests that maternal indomethacin causes ductal constriction in many fetuses.

In the present randomized study, we evaluated the ductal flow velocities and the hemodynamic signs of cardiac failure before, during, and after administration of either indomethacin or β-sympathomimetic tocolytic therapy during spontaneous premature labor. Our aim was to study whether ductal constriction, as defined by echocardiographic flow velocities, was specifically associated with indomethacin, and whether the degree of ductal constriction depends on the length of gestation.

Material and methods

Twenty-seven women with threatened premature birth were enrolled for the study in the University Central Hospital, Helsinki, between February 1988 and May 1989 (Table I). The inclusion criteria were as follows: uterine contractions at least 10-minute intervals for at least 2 hours between 24 and 34 weeks' gestation in singleton pregnancy; documented cervical changes (softening, opening); intact fetal membranes; absence

From Children's Hospital[a] and the Departments of Obstetrics and Gynecology,[b] University of Helsinki, and the Department of Pediatrics, University of California, Irvine.[c]
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Reprint requests: Dr. Marianne Eronen, Children's Hospital, Stenbackinkatu 11, Helsinki, Finland 00290.
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Table I. Clinical characteristics of the study populations

	Indomethacin group	*Nylidrin group*
No. of women	14	13
Maternal age (yr)*	30.1 ± 5.8	28.9 ± 4.8
Gestational age at study (wk)*	28.5 ± 3.2	30.3 ± 2.9
Duration of therapy (hr)*	39.5 ± 21.6	34.2 ± 18.1
Gestational age at birth (wk)*	36.1 ± 5.5	34.2 ± 4.1
Birth weight (gm)*	2602 ± 1125	2481 ± 931
Apgar scores†		
1 minute	7.6 (2-10)	7.8 (3-10)
5 minute	9.1 (4-10)	9.1 (6-10)

*Mean ± SD.
†Median (range).

of intrauterine growth retardation, chorioamnionitis, placenta previa, maternal hypertension, sensitivity to salicylates, or history of peptic ulcer. None of the pregnant women received glucocorticoid treatment. The patients signed informed consent forms before participation in the study.

The patients were randomly allocated (a sealed envelope) to receive either indomethacin or nylidrin hydrochloride. The treatments were identical in appearance so that each patient received intravenous infusion (physiological saline solution) containing either nylidrin (13 patients; initial dose of 50 μg/min to maximal dose of 150 μg/min) or placebo, and rectal suppositories containing either placebo or 100 mg of indomethacin (14 patients); suppository treatment was continued with placebo capsules or capsules containing indomethacin 50 mg every 8 hours for a total of 72 hours.

The fetal heart was studied with two-dimensionally guided, pulsed, and continuous-wave Doppler equipment (Advanced Technology Laboratories 600 MK or Toshiba SSH 65 A Color Doppler system; 3.75 and 5 MHz) before the start of the therapy and 12 and 24 to 36 hours later. In addition, a scan was done 24 and 72 hours after discontinuation of therapy. Two-dimensional imaging was obtained from four approaches including four-chamber, long-axis, short-axis, and arch views. The ductal arch was obtained either in longitudinal or in short-axis view, depending on the orientation of the fetus. The aortic arch was identified in each case to avoid it being mistaken for the ductal arch. When measuring ductal velocities the sampling cursor was placed at the junction of the ductus and the descending aorta until the maximum velocity was attained.[19] Increase in the flow velocity indicates a narrowing of the vessel. In this study, ductal constriction was defined as the presence of a systolic velocity of >140 cm/sec in conjunction with a diastolic velocity of >35 cm/sec. Pulmonary artery flow velocities from the main pulmonary artery were recorded after withdrawal from the site of sampling from the ductus. The angle between the Doppler cursor and the direction of flow was always <15 degrees. Sample volume size was 0.1 to 0.3 cm. At least five waveforms were measured each time on the screen, and the mean of these values was recorded. The variation coefficient of velocities ranged between 11% and 23%.

Total cardiac dimension was measured by determining the distance between the anterior wall of the right ventricle and the posterior wall of the left ventricle at the level of both atrioventricular valves by four-chamber view.[20] The tricuspid valve regurgitation was visualized as a holosystolic regurgitant flow in the four-chamber view by pulsed, continuous-wave, and color Doppler ultrasonography. Fetal heart rate was registered. All studies were recorded on videotape for later playback and analysis.

The results are expressed as means ± SDs. The changes in the flow velocities were evaluated by the analysis of variance (repeated measurements) and the two-tailed, paired Student t test. To compare the velocities between the two tocolytic groups, an unpaired t test was used. The significance of the relationship between the ductal flow velocity and the gestational age was evaluated (Pearson's correlation coefficient). A p value of <0.05 was considered significant.

Results

There were no differences in clinical variables between the women who received indomethacin and those who received nylidrin (Table I). Indomethacin postponed the delivery on average by 7.6 weeks (SD, 5.8), nylidrin by 3.9 weeks (SD, 4.4) ($p = 0.07$). Of the 27 neonates, five required mechanical ventilation; two of them were in the indomethacin group. One infant (25 weeks' gestation; birth weight, 680 gm) died of streptococcus group B sepsis.

Recording of base-line echocardiograms failed in seven mothers because of maternal discomfort. Post-therapy blood flow measurements could not be obtained in six fetuses because of preterm delivery. The treatment was discontinued in one case because fetal echocardiography, performed after the first dose of indomethacin, revealed tetralogy of Fallot with the possibility of ductal-dependent pulmonary circulation.

In the indomethacin group the systolic velocities in the ductus arteriosus were higher than those in the nylidrin group (Table II). The difference in systolic velocities was evident at the 12-hour ($p = 0.003$) and the 24- to 36-hour ($p = 0.03$) echocardiogram. The systolic velocity exceeded 140 cm/sec in nine of the fetuses in the indomethacin group (Fig. 1). The diastolic flow velocities in the ductus were higher during indomethacin treatment than during nylidrin treatment ($p = 0.0008$); and the difference was significant at both the 12-hour ($p = 0.004$) and the 24- to 36-hour

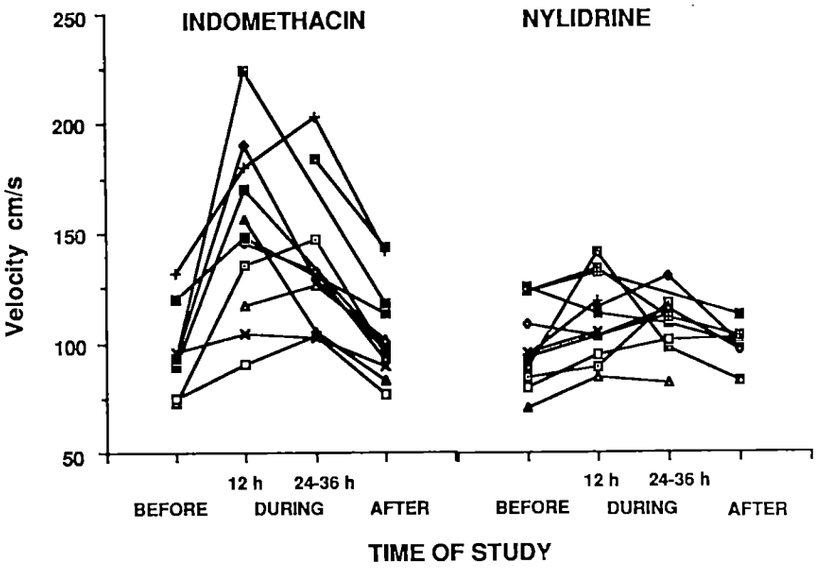


Fig. 1. Peak systolic velocities in fetal ductus before, during (at 12 hours and 24 to 36 hours), and after tocolytic therapy.

Table II. Flow velocity values in the fetal ductus in indomethacin and nylidrin groups

	Indomethacin			*Nylidrin*			
	Mean	*SD*	*Range*	*Mean*	*SD*	*Range*	*p Value*
Values before treatment							
No. of cases	8	—	—	11	—	—	—
Ductus arteriosus							
Peak systolic velocity (cm/sec)	96.4	20.4	73-132	99.6	19.1	71-126	0.73
Diastolic velocity (cm/sec)	20.4	2.2	18-25	21	4.4	16-31	0.72
Pulmonary artery peak velocity (cm/sec)	63.1	9.3	52-75	66.5	8.5	56-86	0.42
Ductal peak velocity/pulmonary artery velocity	1.5	0.2	1.2-1.8	1.5	0.2	1.2-1.9	0.8
Highest values during treatment							
No. of cases	13	—	—	13	—	—	—
Ductus arteriosus							
Peak systolic velocity (cm/sec)	156.6	36.6	103-224	115.9	17.0	85-142	0.001
Diastolic velocity (cm/sec)	52.0	27.5	18-116	22.3	4.4	16-30	0.0008
Pulmonary artery peak velocity (cm/sec)	68.5	12.9	41-91	70.7	8.3	62-92	0.59
Ductal peak velocity/pulmonary artery velocity	2.3	0.34	1.8-2.7	1.6	0.2	1.2-1.9	0.0001
Values after treatment							
No. of cases	12	—	—	7	—	—	—
Ductus arteriosus							
Peak systolic velocity (cm/sec)	106.8	19.6	83-144	103.1	15.7	83-135	0.68
Diastolic velocity (cm/sec)	21.5	3.1	16-27	18.4	2.6	15-22	0.04
Pulmonary artery peak velocity (cm/sec)	68.6	13.7	52-93	71.6	13.6	52-89	0.64
Ductal peak velocity/pulmonary artery velocity	1.6	0.3	1.1-2.3	1.5	0.3	1.1-1.8	0.4

($p = 0.01$) echocardiogram. An increase in the diastolic velocity to >35 cm/sec was noticed in 10 of the fetuses in the indomethacin group (Fig. 2). Nine fetuses in the indomethacin group fulfilled the criteria of ductal constriction[19] when studied on average during 29.2 gestational weeks (range, 26.3 to 34 weeks). In the fetuses without ductal constriction, the mean gestational age was 25.5 weeks (range, 24.3 to 26.6 weeks; $p < 0.05$).

In the nylidrin group only one fetus (29 weeks' gestation) had a clearly elevated systolic velocity in the

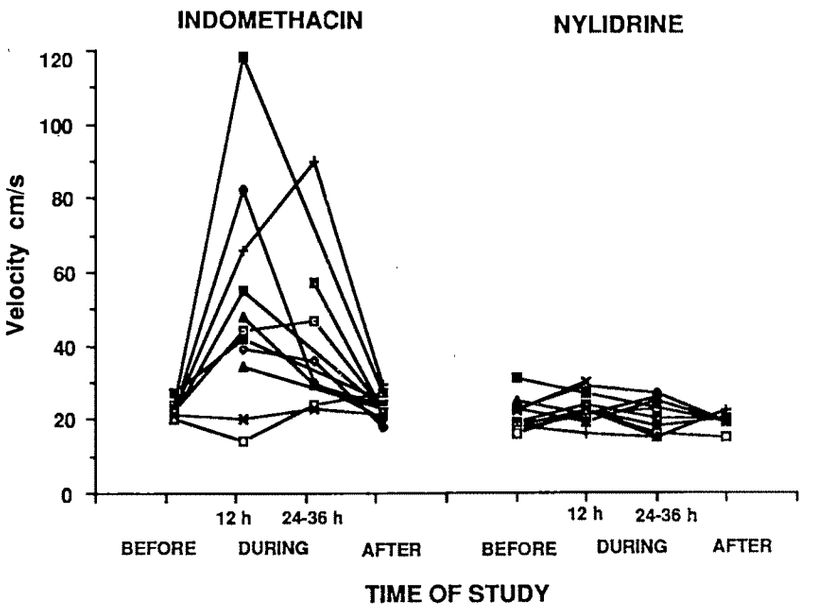


Fig. 2. Diastolic velocities in fetal ductus before, during (at 12 hours and 24 to 36 hours), and after tocolytic therapy.

ductus during treatment (baseline, 90 cm/sec to 142 cm/sec; fetal heart rate, 135 to 155 beats/min). None of these fetuses had a diastolic velocity of >35 cm/sec (range, 16 to 30 cm/sec). In this group three fetuses had atrial ectopic beats during therapy. The mean heart rate during nylidrin administration was significantly higher than during administration of indomethacin ($p = 0.002$).

A significant increase in ductal flow velocities occurred in both groups at the 12-hour echocardiogram. In the indomethacin group the change was significant in both systolic and diastolic velocities ($p = 0.006$ and $p = 0.01$; $n = 8$), whereas the nylidrin group only the systolic velocity increased ($p = 0.04$; $n = 11$). There were no statistically significant differences in the flow velocity values between the two measurements (at 12 and 24 to 36 hours) during the therapies with tocolytic agents.

There were no differences in pulmonary artery flow velocities between the groups ($p > 0.5$). The ratio of peak ductal/pulmonary artery flow was significantly higher in the indomethacin group than in the nylidrin group ($p = 0.0001$) (Table II).

Tricuspid valve regurgitation was detected in three fetuses. The mothers received indomethacin at 29, 32, and 34 weeks' gestation. Base-line echocardiograms were normal. The mean ductal systolic velocity in these fetuses was 204 cm/sec (range, 184 to 224 cm/sec) and mean diastolic velocity 86 cm/sec (range, 55 to 116 cm/sec). In one fetus (32 weeks' gestation) with the highest systolic (224 cm/sec) and diastolic (116 cm/sec) velocities, indomethacin was discontinued after two doses because of evidence of cardiac failure. The tricuspid regurgitation resolved within 24 hours after treatment in two fetuses and within 72 hours in one fetus (34 weeks' gestation).

The total cardiac dimensions increased by 8% ± 6% in fetuses with ductal constriction ($p = 0.02$). In fetuses with ductal constriction accompanied by tricuspid regurgitation, the cardiac dimensions increased by 19% ± 1%. Fetal echocardiograms 3 days after cessation of the treatment were normal in both groups (Figs. 1 and 2).

To evaluate whether the indomethacin-induced increase in ductal flow velocity was related to the length of gestation, the percentage increases in the ductal flow velocities were calculated. There was a significant positive relationship between the gestational age and the change in ductal systolic velocity ($r = 0.65$, $p = 0.01$, $n = 8$ Fig. 3). A less marked relationship was seen in diastolic velocity ($r = 0.42$, $p = 0.08$, $n = 8$, Fig. 3). There was no detectable relationship between the ductal flow velocities and the gestational age in the absence of indomethacin ($r = 0.004$, $p > 0.5$).

Comment

In the present randomized trial, it has been shown that indomethacin given during spontaneous preterm labor constricts the fetal ductus in nine of the 14 fetuses studied. The diagnosis was made on the basis of elevation of peak systolic and diastolic velocities after drug administration.[19] According to a study on fetal lambs

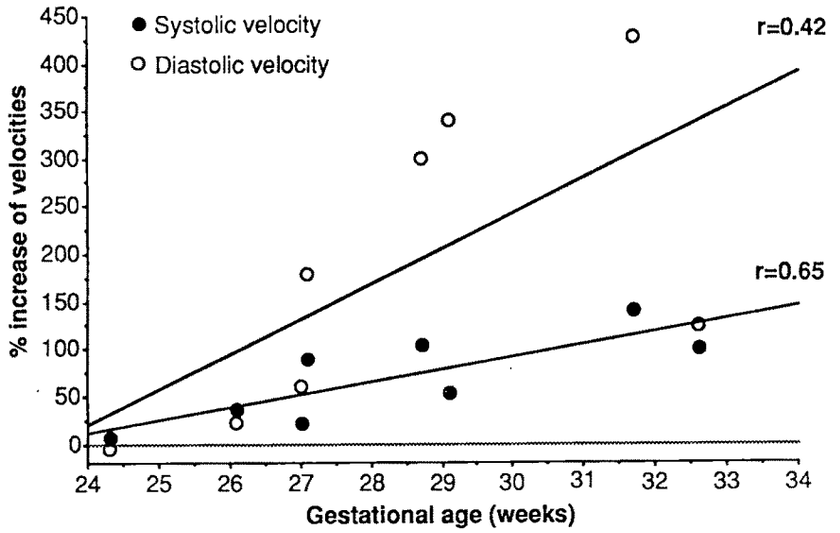


Fig. 3. Percentage increases of systolic and diastolic velocities in fetal ductus during maternal indomethacin treatment.

undergoing mechanical constriction of the ductus arteriosus, the square of peak systolic and diastolic velocities assessed by Doppler echocardiography correlated well with the pressure gradient across ductus arteriosus. Therefore increased ductal flow velocities reflect the constriction of the fetal ductus arteriosus. Our findings are supported by similar results from other groups.[18, 19]

Maternal indomethacin or nylidrin treatment did not affect the pulmonary artery flow velocities. This does not rule out the possibility of an indomethacin-induced increase in pulmonary vascular resistance[10] because a simultaneous increase in pulmonary arterial pressure may balance the pulmonary arterial flow velocity. However, according to the experimental study[10] an increase in pulmonary vascular resistance first took place after several days of surgical ligation of ductus arteriosus. In our study the reversibility of the ductal flow velocity after discontinuation of the drug suggests that the circulatory changes were reversible.

According to a recent study,[18] there was no correlation between the closure of the fetal ductus and the gestational age. In the present investigation the ductal constriction took place as early as 26 weeks' gestation and as late as 34 weeks' gestation. In addition, there was a significant linear positive relationship between the gestational age and the change in ductal flow velocity. The mean gestational age of the fetuses with ductal constriction was also higher than those without constriction. These findings may indicate that the ductus becomes more reactive to indomethacin with increasing gestational age.

Cardiac failure, as evidenced by cardiomegaly and tricuspid valve incompetence, is a severe complication of ductal constriction during fetal life. Elevated pressure in the right ventricular outflow tract may lead to endocardial ischemia with papillary muscle dysfunction.[9] In our study tricuspid regurgitation suggestive of heart failure developed in three of 14 fetuses in the indomethacin group. The ductal flow velocities (systolic mean, 203.7 cm/sec; diastolic mean, 86.3 cm/sec) were the highest recorded during the indomethacin treatment. In each case withdrawal of indomethacin abolished the tricuspid insufficiency within 72 hours. The range of the gestational ages of these fetuses—from 29 to 34 weeks—indicates that the risk of this serious complication is to be taken into consideration when prostaglandin inhibitors are used in premature labor and that indomethacin treatment might be safer in fetuses of <29 weeks' gestation.

Indomethacin-induced ductal constriction may impede either the pulmonary or systemic circulation in cases of cardiac anomalies with a ductus-dependent circulation. These malformations are, for example, tetralogy of Fallot, pulmonary atresia or stenosis, and coarctation or interruption of the aortic arch. One fetus in our study had tetralogy of Fallot. Indomethacin treatment was discontinued because of the risk of ductus-dependent pulmonary circulation. The female infant was born 1 day after administration of indomethacin at 34 weeks' gestation and had Apgar scores of 9. At 5 months of age a Blalock-Taussig shunt was performed because of hypoplastic pulmonary arteries.

In view of our study it is prudent to recommend careful evaluation of the risk/benefit ratio in the use of indomethacin during preterm labor. However, this drug seems to be more effective than ritodrine[11] or nylidrin in arresting preterm contractions, as evidenced

by the present data. In the present study the short course of indomethacin was not given when the mother was exposed to other drugs, which might potentiate ductal constriction (for example, glucocorticoids[21]), thus increasing the risk of cardiac failure. We recommend that ultrasonographic examination of the fetal heart be performed when indomethacin is used. Doppler ultrasonographic examination should include evaluation of the cardiac anatomy and atrioventricular valve function as well as the measurements of ductal flow velocities and the total cardiac dimension. Further studies are under way to identify the effect of indomethacin in the prevention of premature birth, the perinatal outcome, and the follow-up of these children.

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A selective serotonin reuptake inhibitor, fluoxetine hydrochloride, modulates the pulsatile release of prolactin in postmenopausal women

Randall J. Urban, MD,[a, b] and Johannes D. Veldhuis, MD[b]
Galveston, Texas, and Charlottesville, Virginia

We investigated serotoninergic regulation of prolactin release in estrogen-withdrawn postmenopausal women by using a serotonin reuptake inhibitor. Subjects underwent frequent blood sampling after placebo (basal) or fluoxetine administration. Mean 24-hour serum prolactin concentrations increased significantly in response to fluoxetine. Objective pulse analysis revealed no change in prolactin pulse frequency with serotoninergic stimulation, but maximal serum prolactin peak heights increased significantly. Multiple-parameter deconvolution disclosed no change in prolactin half-life, but a significant increase in the total mass of prolactin secreted per 24 hours during fluoxetine administration. Cosinor analysis of the prolactin time series showed a significant increase in the circadian amplitude and mean without any change in the time of maximal concentration during treatment with fluoxetine. We conclude that short-term activation of the serotoninergic system in the absence of substantial estradiol and opiatergic tone significantly increases the secretion of prolactin in postmenopausal women. (AM J OBSTET GYNECOL 1991;164:147-52.)

Key words: Prolactin, serotonin, pulsatile release, fluoxetine

Under physiologic conditions, prolactin is secreted by the anterior pituitary gland in discrete pulses.[1, 2] The exact neuroendocrine mechanisms that control the frequency and amplitude of pulsatile prolactin release are not so well characterized as those that subserve other anterior pituitary hormones, such as the gonadotropins.[3]

The neurotransmitter chemical serotonin seems to participate in the regulation of prolactin secretion in man.[4] Early studies in human beings conflicted on whether pharmacologic probes that increased central nervous system serotonin levels stimulated prolactin release from the anterior pituitary gland.[5-7] Recently, studies in normal men and menstruating women showed that agents presumptively resulting in the elevation of central nervous system serotonin concentrations also increase mean serum prolactin concentrations.[8-10] Moreover, animal studies have shown that estradiol may play a stimulatory role in prolactin release in part by acting through endogenous opioid peptide pathways that activate release of serotonin.[11-15]

To assess the effects of putatively increased central nervous system serotonin levels in the absence of substantial estradiol and opiatergic tone, we studied the pulsatile secretory dynamics and clearance rates of prolactin in estrogen-deficient postmenopausal women basally and after treatment with a selective serotonin reuptake inhibitor, fluoxetine hydrochloride.[16, 17] Significant increases in serum prolactin concentrations with serotoninergic stimulation would indicate that direct or indirect stimulatory effects of serotonin can activate prolactin release in the absence of major influences of estradiol or the opiatergic pathway.

Methods

Patient characteristics. Seven healthy postmenopausal women (ages 53 to 61 years) with no acute or chronic illness, of normal body weight, and taking no medications participated in this study after provision of written informed consent that was approved by the Human Investigation Committee of the University of Virginia Health Sciences Center. Each subject had normal serum concentrations of thyroxine and prolactin, and normal results of biochemical tests of metabolic, hepatic, renal, and hematologic function. Any prior

From the Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Texas at Galveston Medical Branch,[a] and the Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Virginia.[b]
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Reprint requests: Johannes D. Veldhuis, MD, Box 202, Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, VA 22908.
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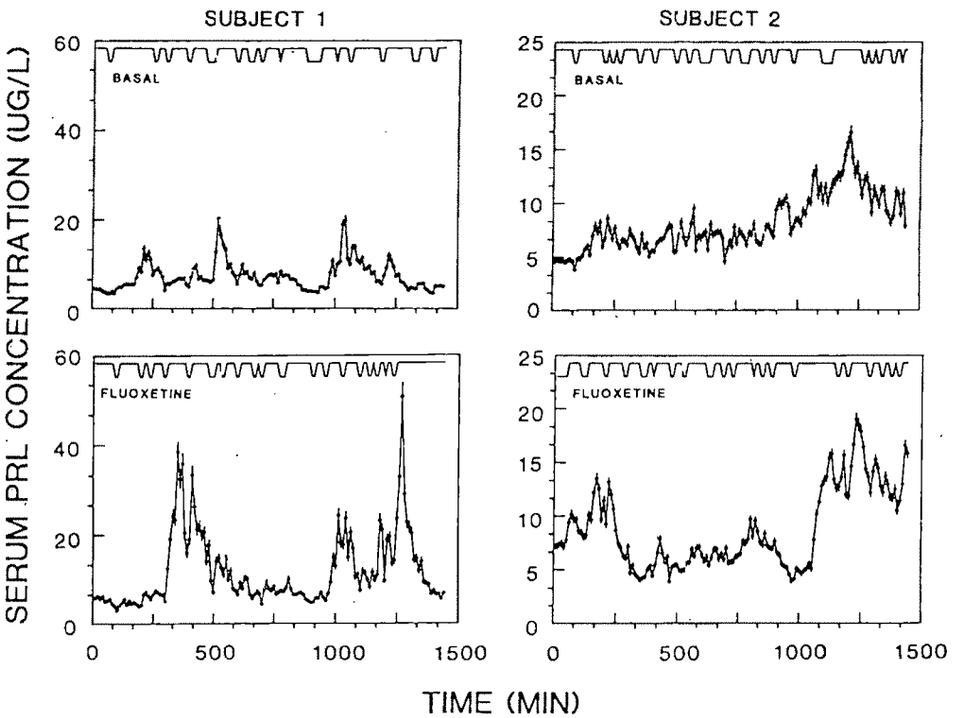


Fig. 1. Twenty-four–hour serum prolactin concentration profiles in two of seven estrogen-withdrawn postmenopausal women basally and during treatment with fluoxetine. Blood was sampled at 10-minute intervals for 24 hours and subsequent serum samples submitted to immunoradiometric assay to determine serial serum prolactin concentrations. *Vertical mark* through each data point denotes intrasample SD (see Methods). Schematized deflections at top of each panel identify significant prolactin pulses detected by Cluster analysis.[24] Zero time on X-axis is clock time of 8:00 AM. Patients were fed at 9:00 AM, 12:00 noon, and 6:00 PM. Lights out for sleeping was stipulated at 11:00 PM.

exogenous estrogens were withdrawn for at least 6 weeks. At the time of study, all women were demonstrably estrogen deficient (serum estradiol concentration <20 pg/ml) with elevated serum gonadotropin concentrations.

Clinical protocol. Volunteers were admitted to the University of Virginia Clinical Research Center on two separate occasions separated by 4 to 8 weeks in a prospective, double-blind, randomized placebo-controlled trial. The subjects received either fluoxetine or a nearly identical placebo pill before each study session. Thus some subjects received fluoxetine before their first admission and placebo before their second admission, whereas others received the treatments in reverse order. Each subject served as her own control and significant treatment differences were sought with the paired Student *t* test (see statistical analysis). Both investigator and subject were blinded to the drug identity. Fluoxetine was used in these preclinical studies under an investigator-initiated investigational new drug approved by the Food and Drug Administration. The drug was administered orally three times daily as 20 mg fluoxetine capsules for 6 days before study as well as during the 24 hours of blood sampling. Because the serum half-life of the drug is 1 to 3 days,[16] this paradigm assured that sampling was done at near steady-state drug levels. The dose of fluoxetine administered is equivalent to that recommended for treatment of depression.[16] The sampling protocol consisted of blood withdrawal (3 ml) every 10 minutes for 24 hours beginning at 8:00 AM from a venous catheter placed in a peripheral arm vein. Subjects were provided three regular meals and allowed to ambulate. An intravenous catheter was inserted at least 1 hour before serial sampling was initiated.

Prolactin assay. Serum samples were assayed in duplicate for prolactin content by a solid phase two-site immunoradiometric assay (Hybritech Inc., San Diego, Calif.). This method has a cross-reactivity of <0.01% with human luteinizing hormone, hu-

man follicle-stimulating hormone, human thyroid-stimulating hormone, human placental lactogen, and human chorionic gonadotropin. The lower limit of sensitivity of this assay is 3.0 μg/L and it has an intraassay coefficient of variation between 3.9% to 4.4% across the range of prolactin values encountered here.

Pulse analysis. An objective computer-based pulse detection method, Cluster analysis, was used to quantitate specific properties of pulsatile prolactin release.[18] To restrict false-negative and false-positive error rates to <10%, the following program parameters were used: (1) test Cluster sizes of two points for nadirs and one point for peaks; (2) pooled Student's *t* test statistics of 2.0 for significant increases and decreases; and (3) a power function model to estimate the individual dose-dependent within-sample variance from all 145 experimental samples in each subject.[1, 3, 18]

Multiple-parameter deconvolution. As described previously in detail,[19] a multiple-parameter deconvolution model was used to analyze the 24-hour serum prolactin concentration profiles basally and after treatment with fluoxetine. This model assumes that plasma hormone concentrations at any given instant are controlled by the combined effects of four secretion and clearance features: (1) locations; (2) amplitudes; and (3) durations of discrete secretory bursts acted on by (4) subject-specific endogenous metabolic clearance.[19] This model estimates the number, amplitude, and duration of all statistically significant underlying secretory events that give rise to the pulsatile plasma hormone concentration profile, and simultaneously calculates a subject-specific hormone half-life.

Cosinor analysis. Statistically based group cosinor analysis of circadian rhythmicity was carried out exactly as described earlier.[20] Briefly, we used simultaneous nonlinear, least squares curve fitting of all data sets and their dose-dependent intrasample variances to compute the group mesor (mean), amplitude, and acrophase (time of maximal concentrations) with conjoint asymmetric statistical confidence limits for the group of women.

Statistical analysis. For Gaussian-distributed measures (means), within-subject differences were sought by paired two-tailed Student's *t* test. Because of departures from normality shown by the Wilks-Shapiro statistic, individual pulse characteristics were compared after logarithmic transformation with subsequent paired two-tailed Student's *t* test.[3] Results are expressed as means ± SEM. Statistical significance was construed for $p \leq 0.05$.

Results

Mean serum prolactin concentrations. The 24-hour mean serum prolactin concentration was significantly increased during treatment with fluoxetine (basal, 9.6 ± 1.6 μg/L vs fluoxetine, 11.1 ± 1.7 μg/L, $p \leq 0.05$).

Table I. Characteristics of specific prolactin pulse properties before and during fluoxetine administration

Pulse characteristics	*Basal*	*Fluoxetine*
Prolactin pulse frequency (pulses/24 hr)	18 ± 1*	18 ± 1
Prolactin interpulse interval (min)	72 ± 8	70 ± 5
Prolactin maximal peak height (μg/L)	11 ± 1	14 ± 1†
Prolactin valley mean concentration	9 ± 1	11 ± 1†

*Mean ± SEM; $n = 7$.
†Statistically significant, $p \leq 0.05$.

Prolactin pulsatility. Fig. 1 shows two of the seven 24-hour prolactin time series basally and after treatment with fluoxetine. Table I summarizes individual values for prolactin pulse characteristics. With fluoxetine treatment, there was a significant increase in the maximal prolactin peak height and prolactin mean interpulse valley concentration. There was no change in prolactin pulse frequency or interpulse interval. Fig. 2 shows values for individual subject's significant prolactin pulse characteristics that were statistically significant (Table II).

Multiple-parameter deconvolution. Fig. 3 depicts the fit of the deconvolution model (calculated curves) and the computed prolactin secretory bursts basally and after treatment with fluoxetine in one woman. As summarized in Table II, there was a significant increase in the mass of prolactin secreted per 24 hours during fluoxetine treatment, but no significant changes in prolactin secretory burst half-duration (duration of the release episode at half-maximal amplitude), prolactin half-life, or interpeak basal secretion (data not shown). The intersecretory burst intervals calculated by the deconvolution method basally and during fluoxetine treatment (basal, 72 minutes ± 9 minutes vs fluoxetine, 64 minutes ± 7 minutes) were similar to those calculated by Cluster analysis (Table I).

Cosinor analysis. Measures of the prolactin circadian rhythmicity basally and after treatment with fluoxetine are summarized in Table III. There were significant increases in the amplitude of the circadian rhythm and the mean about which the rhythm oscillates (mesor) during fluoxetine treatment. There was no change in the time when the maximal value of the circadian rhythm occurred (acrophase).

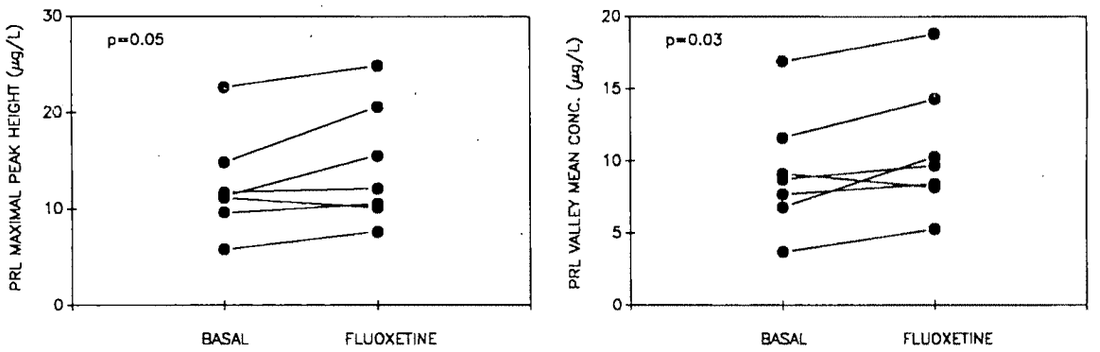


Fig. 2. Individual subject values for significant serum prolactin pulse characteristics, viz., maximal peak height *(left panel)*, and interpeak valley mean serum prolactin concentration *(right panel)* basally and during treatment with fluoxetine.

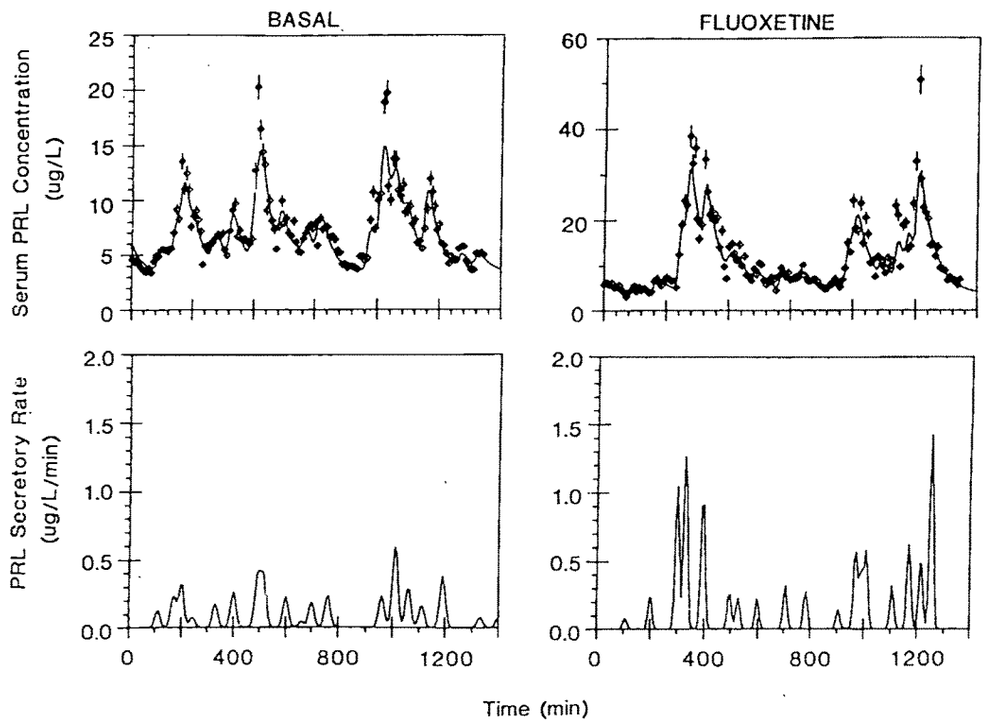


Fig. 3. Reconvolution fit (calculated curve through observed data) and computed prolactin secretory bursts basally and during treatment with fluoxetine in one subject. Fitted continuous curve gives deconvolution-calculated serum prolactin concentrations. Distinct bursts are secretory events computed to give rise to serum prolactin pulse profile.[19]

Comment

We investigated the effects of the selective serotonin reuptake inhibitor, fluoxetine, on the pulsatile secretion and metabolic clearance of prolactin in estrogen-deficient postmenopausal women. We found that presumptive stimulation of serotoninergic pathways increases serum prolactin concentrations in this clinical paradigm (decreased estradiol and low opiatergic tone), and does so by modulation of prolactin secretory burst amplitude without affecting prolactin pulse frequency or its circadian acrophase.

The increased mean serum prolactin concentrations observed after serotoninergic stimulation in this study are consistent with results reported for other agents

previously,[5-10] although the other agents were not so selective as fluoxetine in stimulation of serotoninergic receptors alone. The rise in serum prolactin concentrations was not so great as that in earlier studies. However, these previous studies were done in the presence of estradiol and opiatergic tone.

Opiates have been shown to stimulate prolactin release in adult rats through a serotoninergic mechanism, and in neonatal rats through a nonserotoninergic mechanism.[13, 14] Moreover, estradiol has been implicated in stimulation of prolactin release through an endogenous opioid peptide system.[11, 12] The importance of ovarian hormones in modifying serotoninergic regulation of prolactin release has been suggested further by studies that delineate differential effects of serotonin on prolactin secretion in the rat in proestrus and late pregnancy.[15]

In the present studies in estrogen-deficient postmenopausal women, we could examine the effects of serotoninergic stimulation in the absence of significant estrogenic and opiatergic tone.[17] Under these endocrinologically defined conditions, we observed selective amplitude modulation of prolactin secretion by serotonin without any significant alterations in prolactin pulse frequency, half-life, or circadian acrophase. The observed increase in the mass of prolactin secreted per burst could be a result of direct or indirect stimulatory effects of serotonin on prolactin secretion at the level of the hypothalamus or the pituitary gland. The present studies do not allow us to infer whether this mechanism also subserves the stimulatory actions of opiates and estrogen on prolactin secretion in man. However, if significant increases in serum prolactin concentrations occur in postmenopausal women who are estrogen-deficient while taking fluoxetine, then the prolactin response in women taking fluoxetine who are not estrogen-deficient (normally cycling women and postmenopausal women receiving estrogen replacement) may be even more pronounced. For example, hyperprolactinemia can raise undue clinical concerns with regard to a prolactinoma or other nondrug-related pathophysiology. Further studies are likely to help delineate the exact nature of putative interactions among estrogen, opiates, and serotonin that participate in the coordinate regulation of prolactin secretion in different clinical settings.

We conclude that serotoninergic stimulation can augment the secretion of prolactin in the absence of significant amounts of estradiol and opiatergic tone in postmenopausal persons.

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Table II. Prolactin secretion and clearance calculations in one representative subject basally and during fluoxetine treatment

Parameters	*Basal*	*Fluoxetine*
Secretory burst half-duration (min)	25 ± 5*	18 ± 3
Prolactin half-life (min)	30 ± 5	38 ± 5
Prolactin daily secretory mass (μg/L/24 hr)	110 ± 15	170 ± 30†

*Mean ± SEM.
†Statistically significant, $p \leq 0.05$.

Table III. Twenty-four hour rhythms in serum prolactin concentrations in seven postmenopausal women studied basally and during treatment with fluoxetine

Parameters	*Basal*	*Fluoxetine*
Circadian amplitude (μg/L)	1.3 ± 0.3*	2.1 ± 0.3†
Time of maximal concentration (acrophase, min before 8:00 AM)	450 ± 20	500 ± 20
Group mean (mesor) (μg/L)	5.0 ± 0.2	6.3 ± 0.2†

*Mean ± SEM; $n = 7$.
†Statistically significant, $p \leq 0.05$.

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Endoscopic sclerotherapy in extrahepatic portal hypertension in pregnancy

David Pauzner, MD,[a] Igal Wolman, MD,[a] David Niv, MD,[b] Amos Ber, MD,[a] and Menachem P. David, MD[a]

Tel-Aviv, Israel

Extrahepatic portal hypertension usually occurs during childhood, but a substantial number of patients may reach adult life. There is a general agreement that pregnancy may become hazardous to these patients, and some authors even consider this condition as a contraindication to conceiving. A case of endoscopic sclerotherapy in such a patient is herein presented, and the approach to such patients is discussed. (AM J OBSTET GYNECOL 1991;164:152-3.)

Key words: Extrahepatic portal hypertension, endoscopic sclerotherapy

Extrahepatic portal hypertension usually occurs during childhood or adolescence. The etiologic processes involve venous obstruction. Since liver function is usually preserved and the reproductive system is seldom affected, an obstetric team is bound to encounter such patients.

From the Departments of Obstetrics and Gynecology "B"[a] and Anesthesiology,[b] Serlin Maternity Hospital, Tel-Aviv Medical Center, Sackler Medical School, Tel-Aviv University.
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Reprint requests: Dr. D. Pauzner, Department of Obstetrics and Gynecology "B," Serlin Maternity Hospital Tel-Aviv, Sourasky Medical Center, 15 Ein dor St., Tel-Aviv 61070, Israel.
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Many authors agree that pregnancy may be hazardous to these patients, as it increases the frequency of variceal bleeding which in some instances may be fatal. A debate exists as to the management of variceal bleeding, whether these patients should conceive, and the mode of delivery.

Here we report two consecutive deliveries of the same patient with extrahepatic portal hypertension, treated by endoscopic sclerotherapy.

Case report

A 23-year-old woman, para 0, gravida 1, was admitted to our ward because of hematemesis in her thirty-fifth week of pregnancy. Her history revealed portal

vein thrombosis verified by angiography at 15 years of age. A splenectomy was performed 2 years later because of variceal bleeding, and esophageal transection was done the following year because of recurrent episodes of variceal bleeding. During the 3 years before this admission she was treated successfully by sclerotherapy because of few bleeding episodes.

On admission blood pressure was 130/80 mm Hg and pulse rate was 76 beats/min. She was in good general condition with no signs of melena or further esophageal bleeding.

Endoscopic examination revealed two thrombotic varices in the lower esophagus, with no sign of ulceration or bleeding. The patient was treated with bed rest and antacids. Two weeks later, because of a sudden increase in blood pressure to 170/120 mm Hg, a low-segment cesarean section was performed, with delivery of a live male infant weighing 2500 gm with Apgar scores of 10 at 1 and 5 minutes. The postoperative period was uneventful.

Eighteen months after delivery, during which time one episode of hematemesis had occurred and was treated by sclerotherapy, she conceived again. An esophageal endoscopic evaluation performed during the first trimester of pregnancy revealed no dilated varices. Her pregnancy progressed uneventfully until the thirty-seventh week of gestation when a rise in blood pressure to 180/120 mm Hg was noted. The pregnancy was terminated by a low-segment cesarean section. She was delivered of a live female infant weighing 2550 gm with Apgar scores of 10 at 1 and 5 minutes. Her postoperative course was uneventful.

Comment

A debate exists in the literature as to the management of the parturient with extrahepatic portal hypertension and the best mode of delivery.

Kampelmakher[1] stated in 1957 that extrahepatic portal hypertension should be regarded as an absolute contraindication to pregnancy. Since than a major shift in opinions has occurred. At present most authors permit vaginal delivery in patients whose condition is stable but require a decompression operation in patients with bleeding before conception. Although theoretically the shunting procedure should be the most successful procedure, poor results are related to the poor condition of the veins available for anastomosis, the large number of collateral veins, and the size of the veins. One should remember that surgery should be indicated only as a last resort when all conservative measures have failed.

Recent years have witnessed a growing interest in endoscopic injection sclerosis. Several studies have confirmed that sclerotherapy controls acute variceal bleeding in 90% to 95% of patients and that it is more effective than balloon-tube tamponade or conservative treatment.[2]

Sclerotherapy usually eradicates the required number of varices and, when it is used repeatedly, a marked reduction in the number of subsequent bleeding episodes occurred.[3]

A full evaluation of the hemodynamic state and liver functions of such patients should be performed, and the presence of esophageal varices should be ruled out via an endoscope. If there are no other contraindications and the patient is generally in good health, she should be allowed to conceive, after having the risks involved explained to her.

If esophageal varices are shown, we suggest endoscopic injection sclerosis before conception and repeated endoscopic evaluation throughout pregnancy (at least once each trimester). Patients with uncontrolled bleeding in spite of endoscopic injection sclerosis should undergo a definitive shunt operation before conceiving.

The follow-up during pregnancy should be the same as for any other high-risk patient with the addition of absolute prohibition of aspirin ingestion and a vigorous treatment of any infection, as both seem to enhance esophageal bleeding.

The patient should be well sedated during the first stage of labor, preferably with an epidural anesthetic–morphine-bupivacaine combination. This method provides long-lasting pain relief with a stable cardiocirculatory state; thus the need for volume expansion before the sympathetic block is avoided.

The patient should not be allowed to strain, and the second stage of labor should be assisted by an instrumented delivery when feasible. Ten units of packed blood and a Blakemore tube should be prepared. Cesarean delivery should be performed for the usual obstetric indications.

Although conception and delivery impose a considerable risk on the patient with extrahepatic portal hypertension, we feel that they should not be contraindicated, although strict antenatal follow-up is mandatory.

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Tables for estimation of individual risks of fetal neural tube and ventral wall defects, incorporating prior probability, maternal serum α-fetoprotein levels, and ultrasonographic examination results

James G. Thornton, MD, Richard J. Lilford, PhD, and Robert G. Newcombe, PhD
Leeds and Cardiff, United Kingdom

Tables are provided for estimation of the risk of open spina bifida in patients who have elevated maternal serum α-fetoprotein levels but normal results on detailed anatomy ultrasonographic scan. Risks are provided for various prior population risks, for various levels of elevated maternal serum α-fetoprotein, and for various assumptions about the sensitivity of ultrasonographic examination. The formula used is based on sequential multiplication of odds with the use of Bayes' theorem, but an arbitrary reduction (to the power of 0.5) in the degree to which ultrasonographic information modifies the odds has been made to allow for the nonindependence of ultrasonography and maternal serum α-fetoprotein tests. The wide range of resulting risks shows that the decision to offer amniocentesis after a negative scan should be individualized. (AM J OBSTET GYNECOL 1991;164:154-60.)

Key words: α-Fetoprotein, ultrasonography, neural tube defect, gastroschisis, exomphalos

Traditionally maternal serum α-fetoprotein (AFP) measurement has been used as a screening test for neural tube defects and ventral wall defects. The finding of maternal serum AFP above a certain cutoff level was an indication for the more specific diagnostic test of amniocentesis, providing no other obvious cause for the elevated maternal serum AFP value (e.g., multiple pregnancy, anencephaly, or wrong dates) was detected by low-level ultrasonographic scan. However, amniocentesis carries a 0.5% to 1% procedure-related abortion rate and the accuracy of ultrasonography has greatly improved with the introduction of high-resolution real-time scanning. This has led some authors[1] to rely exclusively on detailed ultrasonographic examination in patients with raised maternal serum AFP levels. It has also been argued[2] that maternal serum AFP screening could be omitted altogether and that detailed ultrasonographic examination is adequate for both screening and diagnosis. Nevertheless many authors[3,4] still recommend maternal serum AFP screening and this test is regaining popularity as a screening test for Down's syndrome and other trisomies.[5] Thus the issue of how to respond to a raised level warrants consideration. The decision to perform amniocentesis or rely exclusively on detailed scan depends on a trade-off between the greater accuracy of the former and the greater safety (in terms of iatrogenic fetal loss) of the latter. Whether the risk of amniocentesis is seen as worthwhile is a value decision that patients will make on the basis of the risks of amniocentesis versus the residual risk of a neural tube defect even if a detailed scan is normal. In this article we calculate the latter risks by statistical methods using reasonable assumptions based on literature review. Since anencephaly can be excluded with certainty by a detailed ultrasonographic scan once suspicion of a neural tube defect has been raised, we refer only to risks of lesser degrees of neural tube defect, namely, open spina bifida. In addition we do not dichotomize maternal serum AFP into "raised" or "normal" values but provide risks at a range of levels.

We have made use of tables for the risks of open spina bifida and abdominal wall defects at different levels of maternal serum AFP for populations with different incidences of open spina bifida.[6,7] These figures exclude cases of wrong gestational age, multiple pregnancy, and anencephaly, which is easily excluded by "low-level" scan. In this article we provide tables of the residual risk of open spina bifida for patients with various raised levels who have had a normal detailed ultrasonographic scan. The tables take into account the different underlying incidences of open spina bifida. Since false-positive results are rare on detailed scan and

From the Department of Obstetrics and Gynaecology, St. James's University Hospital, Leeds, and the Department of Medical Computing and Statistics, University of Wales College of Medicine, Cardiff.
Received for publication March 28, 1990; revised July 30, 1990; accepted August 24, 1990.
Reprint requests: James G. Thornton, Senior Lecturer, Department of Obstetrics and Gynaecology, St. James's University Hospital, Leeds LS9 7TF, United Kingdom.
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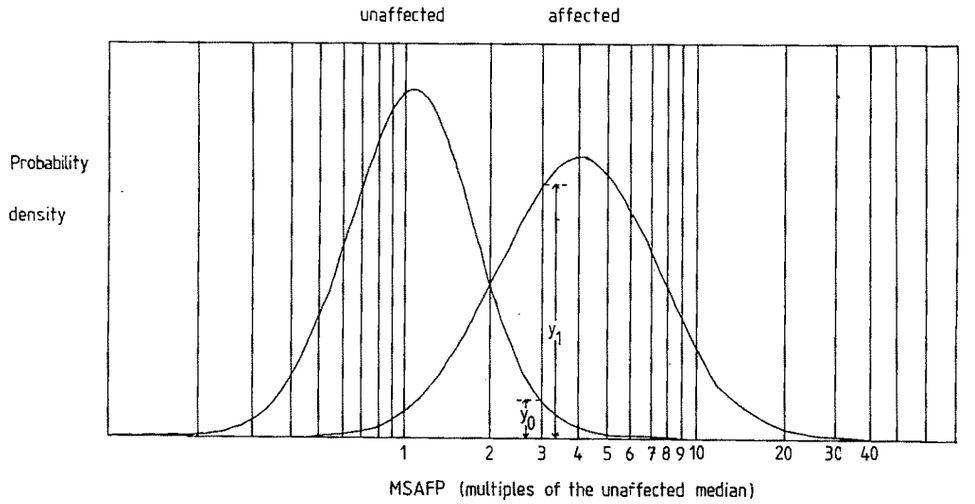


Fig. 1. Distribution of concentrations of maternal serum AFP in unaffected pregnancies and in pregnancies in which the fetus has open spina bifida. The *x* axis represents the gestational age–corrected concentration of maternal serum AFP plotted in multiples of the unaffected median. The y axis represents the probability density of values of multiples of the median with each population. Relative probability densities of affected (y_1) and unaffected pregnancies (y_0) at 3 multiples of the median are shown as an example.

since equivocal findings are likely to be excluded subsequently by amniocentesis, we have confined our attention to the predictive power of an apparently satisfactory normal ultrasonographic examination result. The tables are expanded to take into account different assumptions about the sensitivity of detailed ultrasonographic scan.

Methods

The risks of open spina bifida were calculated on the basis of the prior probability of open spina bifida (the population incidence before any diagnostic tests are performed), the level of maternal serum AFP (treated as a continuous variable), and the result of ultrasonographic examination. The population risk of ventral wall defect was taken to be a constant, 1 in 2000.[8] The recognized differences in population risk of open spina bifida were taken into account by giving posterior risks corresponding to various population risks ranging from 0.1 to 25 per 1000. Rates of open spina bifida in the United States have been published for different geographic areas and racial groups.[9] They range from 0.1 per 1000 for black women in the northwestern states to 1.0 per 1000 for white women in the southeast. The risks of open spina bifida and ventral wall defects in terms of maternal AFP levels were taken from Adams et al.[6] and expressed as likelihood ratios or relative risks.

The formula for calculating posterior odds, given a population incidence (prior odds) and the level of maternal serum AFP, is based on Bayes' theorem. The posterior odds against open spina bifida are calculated by multiplying the prior odds by the likelihood ratio.

	Affected	*Unaffected*
Prior odds	r	$1 - r$
AFP likelihood ratio	y_1	y_0
Posterior odds	ry_1	$y_0(1 - r)$

Posterior odds = Prior odds × Likelihood ratio

$$\frac{\text{Unaffected}}{\text{Affected}} = \frac{(1 - r)}{r} \times \frac{y_0}{y_1}$$

where r is the prior probability of neural tube defect (usually the population incidence) and Y_0 and y_1 denote ordinates or probability densities at the observed log maternal serum AFP values derived from distributions in unaffected and affected samples (Fig. 1).

This process is readily extended to take into account the value of a negative anatomic ultrasonographic result. The posterior odds become $\lambda\, y_0\, (1 - r)$ to $y_1 r$, where λ represents the negative diagnostic value of the scan (i.e., the likelihood ratio of a negative scan).

Assuming the scan had a false-negative rate, β, and a false-positive rate, α, we would have a likelihood ratio of $\lambda = (1 - \alpha)/\beta$. The error rates, α and β, will depend on the technical skill exercised and the level of tolerance of the ultrasonographer for suboptimal views of the spine. The λ value depends more dramatically on β than on α. Let us assume (somewhat arbitrarily and conservatively) that $\alpha = 0.05$. For values of β we consider three plausible false-negative rates: $\beta = 0.2$, 0.1, and 0.05. These would give λ values of 0.95/0.2 (4.75), 0.95/0.1 (9.5), and 0.95/0.05 (19), respectively.

Table I. Parameters of distributions of concentrations of maternal serum AFP in specimens with dates assigned by ultrasonography

	Geometric mean (multiples of median)	*Log_{10} multiples of median (mean ± SD)*
16-18 wk		
Unaffected	1.02	0.0065 ± 0.2050
Open spina bifida	5.42	0.7344 ± 0.2607
Ventral wall defect	8.65	0.9369 ± 0.6610
19-21 wk		
Unaffected	1.02	0.0065 ± 0.1710
Open spina bifida	3.17	0.5005 ± 0.2521
Ventral wall defect	8.65	0.9369 ± 0.6610

However, this simple multiplicative model, known as "independence Bayes'," is inappropriate here since it assumes that the information given by the ultrasonographic scan is of entirely independent discriminatory power to that provided by the maternal serum AFP value. This is unlikely to be the case since larger lesions are more likely to show up with either method. Independence Bayes' will therefore assign excessively polarized risks. We get around this by using a likelihood ratio less extreme than $(1 - \alpha)/\beta$ to modify the prior odds $y_0 (1 - r) : y_1 r$ into the posterior odds. Exactly how this should be done could be definitely established only by a very large study examining the relationship of maternal serum AFP and ultrasonography jointly to the true state of the fetus. This would be beyond our scope and likely also beyond that of others. A single-center study would need many years to accrue sufficient data, and technical improvements in ultrasonography would probably supersede the results before they were available. In a multicenter study it would be difficult to allow for heterogeneity in effective α and β rates between centers. In the absence of hard data, we try to devise a means of lengthening the odds but to a lesser degree than an independence Bayes' model.

We use

$$\frac{1 - r}{r} \times \frac{y_0}{y_1} \times \left(\frac{1 - \alpha}{\beta}\right)^{\gamma}$$

as our posterior odds, for some power γ between 0 (ignoring ultrasonography and assuming it gives identical information to maternal serum AFP) and 1 (assuming complete independence). The γ value depends on the degree of nonindependence; in the absence of directly pertinent data, we arbitrarily choose a location parameter of one half for our distribution of degrees of belief regarding γ. When this is done, the λ values corresponding to $\beta = 0.2$, 0.1, and 0.05 are reduced to 2.179, 3.082, and 4.359, respectively. It must be emphasized that the assumption that γ is one half as a measure of the degree of nonindependence of maternal serum AFP and ultrasonographic examination is arbitrary. Because many readers may be unhappy at this choice of γ, we also provide risk figures for cases in which ultrasonography provides no new information and where ultrasonography is completely independent of maternal serum AFP.

Quoted rates for sensitivity of ultrasonography for diagnosis of neural tube defect defect (assuming anencephaly has been excluded) range from 60% to 96%.[3, 10-12] The higher figure refers to centers of excellence but may be achievable in average examination units if the ultrasonographer has been alerted to the possibility of neural tube defects by the maternal serum AFP level. We therefore provide three risks assuming ultrasonographic sensitivity rates of 80%, 90%, and 95%. These correspond to false-negative rates, β, of 0.2, 0.1, and 0.05, respectively. There are no widely quoted rates for the specificity of ultrasonographic scan since for large, obvious lesions the false-positive rate will approach 0 and for doubtful lesions confirmation will usually be sought by amniocentesis before termination of pregnancy. We have therefore assumed a false-positive rate for ultrasonography of 5%, which is probably an overestimate but makes allowance for those cases where a satisfactory scan is impossible because of technical difficulties such as maternal obesity and posterior fetal position. Any overestimate will tend to reduce the likelihood ratio, λ, by which we modify the prior odds but the effect is very small.

The data relating maternal serum AFP to open spina bifida used in calculating posterior risks come from an updated version of the data Adams et al.[6] prepared as an unpublished supplement in February 1984, in which confirmation of dates and exclusion of multiple pregnancy and anencephaly by low-level ultrasonography are assumed already to have taken place. The parameters of the distributions of concentrations of maternal serum AFP are shown in Table I. The distributions of concentrations of maternal serum AFP for normal pregnancies and pregnancies affected by neural tube defects come from data reported by Wald et al.[13] for pregnancies where the gestational age was corrected for ultrasonographic results. Such data are not available for ventral wall defect–affected pregnancies, and therefore the parameters have been calculated from last menstrual period data by assuming that the distribution is shifted upward by 2%, the percentage of the shift found in unaffected pregnancies. In Tables II to IV, the risks of open spina bifida and ventral wall defect are reported for three assumptions about ultrasonography, namely, normal detailed anatomic scans of 80%, 90%, and 95% sensitivity, respectively.

Table II. Risks (1 in *n*) of open spina bifida and ventral wall defect by population incidence and maternal serum AFP at 16 to 18 weeks' gestation in patients who have had normal ultrasonographic examination of 80% sensitivity

AFP multiples of median	*Ventral wall defect only*	*Prior incidence of open spina bifida per 1000*								
		0.1	*0.2*	*0.4*	*0.8*	*1.0*	*2.0*	*3.5*	*5.0*	*25.0*
2.0	3600.0	18000.0	9000.0	4500.0	2300.0	1800.0	900.0	510.0	360.0	71.0
	8000.0	**39000.0**	**20000.0**	**9800.0**	**4900.0**	**3900.0**	**2000.0**	**1100.0**	**780.0**	**150.0**
	17000.0	86000.0	43000.0	21000.0	11000.0	8600.0	4300.0	2400.0	1700.0	340.0
2.5	1500.0	4700.0	2400.0	1200.0	590.0	470.0	240.0	140.0	95.0	19.0
	3200.0	**10000.0**	**5100.0**	**2600.0**	**1300.0**	**1000.0**	**510.0**	**290.0**	**210.0**	**41.0**
	6900.0	22000.0	11000.0	5600.0	2800.0	2200.0	1100.0	640.0	450.0	89.0
3.0	590.0	1500.0	740.0	370.0	190.0	150.0	75.0	43.0	31.0	6.8
	1300.0	**3200.0**	**1600.0**	**810.0**	**400.0**	**320.0**	**160.0**	**93.0**	**65.0**	**14.0**
	2800.0	7000.0	3500.0	1800.0	880.0	710.0	350.0	200.0	140.0	28.0
3.5	250.0	530.0	270.0	130.0	68.0	54.0	28.0	16.0	12.0	3.1
	540.0	**1200.0**	**580.0**	**290.0**	**150.0**	**120.0**	**59.0**	**34.0**	**24.0**	**5.5**
	1200.0	2500.0	1300.0	630.0	320.0	250.0	130.0	73.0	51.0	11.0
4.0	110.0	210.0	110.0	54.0	28.0	22.0	12.0	7.1	5.2	11.8
	240.0	**460.0**	**230.0**	**120.0**	**59.0**	**47.0**	**24.0**	**14.0**	**10.0**	**2.8**
	510.0	1000.0	510.0	250.0	130.0	100.0	51.0	30.0	21.0	4.9
4.5	50.0	93.0	47.0	24.0	13.0	10.0	5.6	3.6	2.8	1.4
	110.0	**200.0**	**100.0**	**51.0**	**26.0**	**21.0**	**11.0**	**6.7**	**5.0**	**1.8**
	230.0	440.0	220.0	110.0	56.0	45.0	23.0	13.0	9.7	2.7
5.0	24.0	44.0	22.0	12.0	6.3	5.3	3.1	2.2	1.9	1.2
	51.0	**94.0**	**48.0**	**24.0**	**13.0**	**10.0**	**5.6**	**3.7**	**2.9**	**1.4**
	110.0	200.0	100.0	52.0	26.0	21.0	11.0	6.8	5.0	1.3

The left-hand column gives risks of ventral wall defect and the other columns give risks of open spina bifida for various prior population incidences. At each level of maternal serum AFP and prior probability of open spina bifida three figures are given. The upper figure assumes that ultrasonography provides no information beyond maternal serum AFP ($\gamma = 0$), the lower figure assumes ultrasonography is completely independent of maternal serum AFP ($\gamma = 1$), and the middle figure in boldface type assumes $\gamma = 0.5$ and is our best estimate of any individual woman's true risk.

Results

Tables II to IV give risks (expressed as 1 in *n* to two significant digits) for ventral wall defect in the left-hand column and for open spina bifida in the other columns for various levels of maternal serum AFP tested at 16 to 18 weeks and for various population incidences of open spina bifida. At each level of maternal serum AFP and prior probability of open spina bifida three figures are given. The top figure assumes that ultrasonography provides no information beyond maternal serum AFP ($\gamma = 0$), the bottom figure assumes ultrasonography is completely independent of maternal serum AFP ($\gamma = 1$), and the middle figure (in boldface type) assumes $\gamma = 0.5$ and is our best estimate of any individual woman's true risk. In Tables II to IV the risks incorporating ultrasonographic information take into account a normal detailed anatomic ultrasonogram of 80%, 90%, and 95% sensitivity, respectively.

The risks for ventral wall defect in the first column of each table assume a constant population incidence of 0.5 per 1000 (Adams et al. Unpublished results, 1984).

Illustrative cases

Case 1. A woman from South Wales, who had a previous affected child, had a maternal serum AFP value of 2.5 multiples of the median at 16 weeks and a normal ultrasonographic scan performed by an experienced ultrasonographer with knowledge of her history and maternal serum AFP level. In this population (previous affected child in a high-incidence area) the prior probability of open spina bifida before any investigations is about 1 in 40 (25/1000) after anencephaly is excluded. Assuming a negative ultrasonographic examination of 95% sensitivity, the risk of open spina bifida is estimated at 1 in 81 (Table IV); therefore the patient decided to undergo amniocentesis.

Case 2. A woman from southeast England, with a previous normal delivery, had a maternal serum AFP level of 5 multiples of the median at 17 weeks and a normal ultrasonographic scan performed in a district hospital by an ultrasonographer trained to detect the banana and lemon signs. This patient's prior probability of open spina bifida, excluding anencephaly, is approximately 1 in 1000.[8] Assuming the ultrasonographic scan is 80% sensitive, her posterior probability after scan and with a very high maternal serum AFP level is estimated at 1 in 10 (Table II). She elected to undergo amniocentesis.

Case 3. A woman from South Wales had a maternal serum AFP value of 2.5 multiples of the median in her

Table III. Risks (1 in *n*) of open spina bifida and ventral wall defect by population incidence and maternal serum AFP in patients who have had normal ultrasonographic examination of 90% sensitivity

AFP multiples of median	*Ventral wall defect only*	*Prior incidence of open spina bifida per 1000*								
		0.1	*0.2*	*0.4*	*0.8*	*1.0*	*2.0*	*3.5*	*5.0*	*25.0*
2.0	3600.0	18000.0	9000.0	4500.0	2300.0	1800.0	900.0	510.0	360.0	71.0
	11000.0	**56000.0**	**28000.0**	**14000.0**	**6900.0**	**5600.0**	**2800.0**	**1600.0**	**1100.0**	**220.0**
	35000.0	170000.0	86000.0	43000.0	21000.0	17000.0	8600.0	4900.0	3400.0	670.0
2.5	1500.0	4700.0	2400.0	1200.0	590.0	470.0	240.0	140.0	95.0	19.0
	4500.0	**15000.0**	**7300.0**	**3600.0**	**1800.0**	**1500.0**	**730.0**	**420.0**	**290.0**	**58.0**
	14000.0	45000.0	22000.0	11000.0	5600.0	4500.0	2200.0	1300.0	890.0	180.0
3.0	590.0	1500.0	740.0	370.0	190.0	150.0	75.0	43.0	31.0	6.8
	1800.0	**4600.0**	**2300.0**	**1100.0**	**570.0**	**460.0**	**230.0**	**130.0**	**92.0**	**19.0**
	5600.0	14000.0	7000.0	3500.0	1800.0	1400.0	700.0	400.0	280.0	56.0
3.5	250.0	530.0	270.0	130.0	68.0	54.0	28.0	16.0	12.0	3.1
	760.0	**1600.0**	**820.0**	**410.0**	**210.0**	**170.0**	**83.0**	**48.0**	**34.0**	**7.4**
	2300.0	5100.0	2500.0	1300.0	630.0	510.0	250.0	150.0	100.0	21.0
4.0	110.0	210.0	110.0	54.0	28.0	22.0	12.0	7.1	5.2	1.8
	330.0	**660.0**	**330.0**	**160.0**	**83.0**	**66.0**	**34.0**	**20.0**	**14.0**	**3.6**
	1000.0	2000.0	1000.0	510.0	250.0	200.0	100.0	59.0	41.0	8.9
4.5	50.0	93.0	47.0	24.0	13.0	10.0	5.6	3.6	2.8	1.4
	150.0	**280.0**	**140.0**	**72.0**	**36.0**	**29.0**	**15.0**	**9.1**	**6.7**	**2.1**
	460.0	880.0	440.0	220.0	110.0	88.0	45.0	26.0	18.0	4.4
5.0	24.0	44.0	22.0	12.0	6.3	5.3	3.1	2.2	1.9	1.2
	72.0	**130.0**	**67.0**	**34.0**	**17.0**	**14.0**	**7.6**	**4.7**	**3.6**	**1.5**
	220.0	410.0	200.0	100.0	52.0	42.0	21.0	13.0	9.1	2.6

For explanation of values see footnote to Table II.

first pregnancy at 17 weeks and a normal detailed ultrasonographic scan, by an experienced radiologist who believes that this sensitivity is 90% when he is alerted to a raised maternal serum AFP value. Her prior probability of open spina bifida was 2 in 1000,[12] and after maternal serum AFP and ultrasonographic scanning the posterior probability of spina bifida is 1 in 730 (Table III) and that of ventral wall defect is 1 in 4500. She elected not to undergo amniocentesis. Note that had the detailed ultrasonographic scan not been performed, she would have been assigned a risk of open spina bifida of 1 in 240 (Table III, upper risk figure) and might well have opted for amniocentesis.

Comment

The major criticism of our calculation is likely to be the assumption of a value of 0.5 for the power γ, by which we reduce the modifying predictive power of a negative ultrasonographic scan to allow for its nonindependence from the maternal serum AFP level. Our defense is that, if the undoubtedly pertinent data that a negative ultrasonographic examination provides are to be used at all in risk revision, then some arbitrary assumption must be made. The necessarily enormous trial in which ultrasonography and maternal serum AFP screening are combined is probably impossible to complete before its findings would be superseded by technical advances in ultrasonography techniques. It may be argued that our value for γ of 0.5 is too low because the two tests, maternal serum AFP and ultrasonography, are closely dependent, with both detecting the larger lesions. We believe that this is unlikely. Although both tests are likely to detect larger lesions, there are other factors besides small size that cause false-negative results and are not linked. False-negative maternal serum AFP results may be caused by laboratory and clerical errors and by skin covering lesions that are usually but not necessarily small. False-negative ultrasonographic results may be caused by operator lapses, faulty identification of anatomy, maternal obesity, or fetal position, none of which is necessarily related to small lesions. In view of this we believe our choice of a value of 0.5 for γ is conservative and, if anything, is likely to underestimate the diagnostic value of a negative ultrasonographic scan. One way of testing the degree of dependence of ultrasonographic examination and maternal serum AFP would be to obtain data on the sizes of lesions detected and missed by both methods. We do not have access to such a data base, but if such information could be obtained elsewhere, a somewhat more objective value for γ could be chosen. Because of our uncertainty about the correct value for γ, we have taken care to present the risks bounded by the range if γ were 0 and if γ were 1. Any individual woman's risk must be within this range, and we believe the figures in boldface type in our tables are reasonable estimates.

Our estimates of 80%, 90%, and 95% sensitivity for ultrasonography may be questioned by some. We believe that these figures are justified for a level II scan

Table IV. Risks of open spina bifida and ventral wall defect by population incidence and maternal serum AFP in patients who have had a negative ultrasonographic examination of 95% sensitivity

AFP multiples of median	*Ventral wall defect only*	*Prior incidence of open spina bifida per 1000*								
		0.1	*0.2*	*0.4*	*0.8*	*1.0*	*2.0*	*3.5*	*5.0*	*25.0*
2.0	3600.0	18000.0	9000.0	4500.0	2300.0	1800.0	900.0	510.0	360.0	71.0
	16000.0	**79000.0**	**39000.0**	**20000.0**	**9800.0**	**7900.0**	**3900.0**	**2200.0**	**1600.0**	**310.0**
	69000.0	340000.0	170000.0	86000.0	43000.0	34000.0	17000.0	9800.0	6800.0	1300.0
2.5	1500.0	4700.0	2400.0	1200.0	590.0	470.0	240.0	140.0	95.0	19.0
	6300.0	**21000.0**	**10000.0**	**5100.0**	**2600.0**	**2100.0**	**1000.0**	**590.0**	**410.0**	**81.0**
	28000.0	90000.0	45000.0	22000.0	11000.0	9000.0	4500.0	2600.0	1800.0	350.0
3.0	590.0	1500.0	740.0	370.0	190.0	150.0	75.0	43.0	31.0	6.8
	2600.0	**6500.0**	**3200.0**	**1600.0**	**810.0**	**650.0**	**320.0**	**190.0**	**130.0**	**26.0**
	11000.0	28000.0	14000.0	7000.0	3500.0	2800.0	1400.0	800.0	560.0	110.0
3.5	250.0	530.0	270.0	130.0	68.0	54.0	28.0	16.0	12.0	3.1
	1100.0	**2300.0**	**1200.0**	**580.0**	**290.0**	**230.0**	**120.0**	**67.0**	**47.0**	**10.0**
	4700.0	10000.0	5100.0	2500.0	1300.0	1000.0	510.0	290.0	200.0	41.0
4.0	110.0	210.0	110.0	54.0	28.0	22.0	12.0	7.1	5.2	1.8
	470.0	**930.0**	**460.0**	**230.0**	**120.0**	**94.0**	**47.0**	**27.0**	**19.0**	**4.6**
	2000.0	4000.0	2000.0	1000.0	510.0	400.0	200.0	120.0	81.0	17.0
4.5	50.0	93.0	47.0	24.0	13.0	10.0	5.6	3.6	2.8	1.4
	210.0	**400.0**	**200.0**	**100.0**	**51.0**	**41.0**	**21.0**	**12.0**	**9.0**	**2.6**
	930.0	1800.0	880.0	440.0	220.0	180.0	88.0	51.0	36.0	7.8
5.0	24.0	44.0	22.0	12.0	6.3	5.3	3.1	2.2	1.9	1.2
	100.0	**190.0**	**94.0**	**48.0**	**24.0**	**20.0**	**10.0**	**6.3**	**4.7**	**1.7**
	440.0	810.0	410.0	200.0	100.0	82.0	42.0	24.0	17.0	4.2

For explanation of values see footnote to Table II.

performed in a well-ordered unit where the ultrasonographer has been alerted to the possibility of open spina bifida by the raised maternal serum AFP level. The middle and lower rows of risks in our tables are *not* applicable if only a low-level dating and fetal counting scan has been performed. Such an examination will probably exclude anencephaly but is unlikely to achieve even 80% sensitivity for open spina bifida. The upper rows of figures in each table would be suitable in this situation if no higher-level scan was possible.

In our analysis of the risk of ventral wall defects we have assumed that ultrasonography has the same sensitivity and specificity as it does in the detection of open spina bifida. Some readers may feel sensitivities of 80% and 90% are underestimates for ventral wall defects and claim that virtually all cases of exomphalos (that is, >98%) will be detected by scan. Although a small gastroschisis is more easily missed, it has a good prognosis and is seldom an indication for prenatal intervention. Such readers may wish to ignore the tables dealing with ventral wall defects and concentrate on the possibility of missing the more common, more serious, and more difficult to detect open spina bifida.

The wide variation in risks of open spina bifida at various maternal serum AFP levels and prior odds indicate that a blanket policy neither of always proceeding to amniocentesis after a raised maternal serum AFP value, whatever the ultrasonographic findings, nor of omitting amniocentesis whenever ultrasonographic examination results are normal, can be justified. The risk assessment and consequent decision must be individualized on the basis of the patient's values and the probabilities. These tables provide a rational and convenient basis for estimation of the probabilities. In particular, because most of the additional cases of neural tube defect detected by amniotic fluid AFP testing will be relatively small and because this is the group from which most severely handicapped long-term survivors come, it is important that a policy of always omitting amniocentesis if the ultrasonographic examination result is normal is not followed. The opposite policy of always performing amniocentesis above an arbitrary maternal serum AFP cutoff of, for example, 2.5 multiples of the median, whatever the ultrasonographic findings and population incidence, is likely to result in high acceptance rates since most patients will have been led to believe that the abnormal maternal serum AFP value put them in a high-risk category. Nevertheless, it will often result in amniocenteses at extremely low fetal risks. These will sometimes be below the original population incidence, occasionally an order of magnitude below the amniocentesis procedure–related abortion rate, and often below the risk that an appropriately informed patient would regard as sufficient to warrant an invasive test.

The calculations given here indicate the considerably lower risks that may be assigned to pregnancies for which a demonstrably normal result has been obtained by ultrasonography. The posterior risk is not 0, because false-negative results do occur, but can be considerably

better than the values given in the tables of Adams et al. We do not consider explicitly here what to do if the scan shows abnormality suggestive but not diagnostic of open spina bifida (e.g., the "lemon" and "banana" frontal and cerebellar signs[14, 15] with no obvious spinal defect); in this case amniocentesis presumably would be recommended anyway, practically irrespective of the maternal serum AFP level.

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Appendix

For users who prefer to calculate individual risks by computer rather than with tables we provide this formula, which can be easily programmed into a portable microcomputer.

y_i = Probability distribution of unaffected ($i = 1$), open spina bifida ($i = 2$), and ventral wall defect ($i = 3$) pregnancies at appropriate AFP multiples of the median

x = Log_{10} multiples of the median

n_i = Prior probability 1 in n of open spina bifida ($i = 2$) or ventral wall defect ($i = 3$)

se = Sensitivity % assumed for ultrasound

sp = Specificity % assumed for ultrasound

γ = 0, 0.5, or 1 (adjustment factor for nonindependence of tests)

r_i = Posterior odds for open spina bifida ($i = 2$) or ventral wall defect ($i = 3$)

μ_i = Mean $\log_{10}$ AFP multiples of the median at appropriate gestation from Table I

σ_i = SD $\log_{10}$ AFP multiples of the median at appropriate gestation from Table I

$$y_i = \left(\frac{1}{\sigma_i}\right)^e - \frac{1}{2}\left(\frac{x - \mu_i}{\sigma_i}\right)^2$$

$$r_i = (n - 1)\left(\frac{y_t}{y_i}\right)\left(\frac{sp}{100 - se}\right)^\gamma$$

If risks are preferred to odds:

Risk of open spina bifida or ventral wall defect = 1 in $r_t + 1$

Risk of open spina bifida and/or ventral wall defect = 1 in $1/(1 - r_2r_3/(1 + r_2)(1 + r_3))$

Heterotopic pregnancies after in vitro fertilization and embryo transfer

Botros Rizk, MD, Seang Lin Tan, MD, Samuel Morcos, MD, Andrew Riddle, MD, Peter Brinsden, MD, Bridgett A. Mason, MB, BS, and Robert G. Edwards, PhD, DSc
London, England

Seventeen cases of heterotopic pregnancies are reported among 1648 clinical pregnancies after in vitro fertilization. The high prevalence of tubal damage among IVF patients and the use of superovulation and multiple embryo transfer might predispose patients to the condition. Nine patients reported abdominal pain and vaginal bleeding, five patients did not have symptoms, and three had acute abdominal emergencies. Transvaginal ultrasonography was superior to transabdominal ultrasonography in the diagnosis of extrauterine pregnancies. The presence of an intrauterine gestation sac in a patient without symptoms should not exclude the diagnosis of a concomitant extrauterine pregnancy until the pelvis is carefully visualized. Early diagnoses of viable ectopic pregnancies before rupture abolishes mortality and morbidity and offers the chance of patient selection for conservative treatment. In two patients the extrauterine gestation sac was treated by transvaginal aspiration and injection of potassium chloride under ultrasonographic guidance. The outcome of the intrauterine pregnancy was favorable regardless of the method of treatment of the ectopic pregnancy. (AM J OBSTET GYNECOL 1991;164:161-4.)

Key words: Heterotopic pregnancy, in vitro fertilization, embryo transfer

The incidence of combined intrauterine and extrauterine pregnancy is evidently higher after in vitro fertilization (IVF) than in spontaneous pregnancies. Our data indicate a frequency of 1% in clinical pregnancies after IVF as compared with 1 in 3889 and 1 in 6778 after spontaneous conceptions.[1,2] Diagnosis of heterotopic pregnancy is difficult because symptoms of ectopic gestation may be attributed to complications of intrauterine pregnancy, and a patient without symptoms may not raise suspicions of ectopic gestation. Transvaginal ultrasonography helps in the diagnosis and should be used in suspected cases, but assays of β-human chorionic gonadotropin (β-hCG), which are usually low in ectopic pregnancies, can be unhelpful inasmuch as they might indicate normal ranges in combined pregnancies.

Management of combined pregnancies presents another difficulty. Laparotomy during the first trimester might threaten the intrauterine pregnancy and ultrasonography-guided aspiration procedures have limitations, although they are valuable in properly selected cases. In the present study we discuss the factors responsible for the increased frequency of combined pregnancy after IVF, the variations in clinical presentation, and the value of transvaginal ultrasonography in early diagnosis and therapy.

From Bourn Hallam Medical Centre.
Received for publication January 10, 1990; revised July 30, 1990; accepted August 3, 1990.
Reprint requests: Botros Rizk, MD, Bourn Hallam Medical Centre, 112 Harley St., London, England W1N 1AF.
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Material and methods

Seventeen cases of combined intrauterine and extrauterine pregnancies were encountered among 1648 clinical pregnancies after IVF at the Bourn Hallam Medical Centre between 1985 and 1989. The methods of ovarian stimulation, ultrasonographic and endocrine monitoring, oocyte retrieval, fertilization of oocytes, and growth of embryos in vitro used in this period have been described previously.[3] Embryo replacements were mostly carried out 2½ days after oocyte retrieval with a catheter (Wallace, Colchester, United Kingdom), and anesthetic agents were not administered to any of the 17 patients. A maximum of four embryos were replaced in 0.02 ml of culture medium deposited near the fundus.

Urinary and serum β-hCG levels were measured 15 days after oocyte recovery. Serial β-hCG levels were measured at 5 days' interval. At Bourn Hall Clinic, transabdominal followed by transvaginal ultrasonography was performed 35 days after oocyte recovery; at Hallam Medical Centre, only transabdominal ultrasonography was performed. Patients returned to the referring obstetricians during the pregnancy and delivery.

Results

Patients and stimulation. The mean age of the 17 patients was 30.5 years (range, 20 to 37 years). Fifteen of them had tubal damage, one had endometriosis with patent tubes, and one had not conceived after repeated donor insemination. Five patients had previous tubal surgery and two had previous ectopic pregnancies.

Ovarian stimulation was achieved by the use of clomiphene citrate (Serophene, Serono Laboratories, Welwyn Garden City, United Kingdom) and human menopausal gonadotropin (hMG; Pergonal, Serono) in 10 patients. Purified follicle-stimulating hormone (FSH; Metrodin, Serono) was added to this regime in two patients. The gonadotropin-releasing hormone (GnRH) agonist (Suprefact, Hoechst, Hounslow, United Kingdom), and hMG were used for stimulation in five patients. The number of oocytes retrieved varied between three and 16, and four embryos were replaced in eight patients, three in seven patients, and two in two patients.

Symptoms and diagnoses. The symptoms at first examination in the 17 patients were quite variable, with abdominal pain and vaginal bleeding in nine patients, acute surgical abdominal emergencies in three, and no symptoms in the remaining five patients (Table I). Fifteen patients had ultrasonographic scans before the definitive management of the extrauterine pregnancy began and the ectopic pregnancy ruptured before ultrasonography in two patients. In 10 patients who had abdominal scans, a correct diagnosis was made in only five and was strongly suspected in one (50%). Each of the five patients who had abdominal and transvaginal scans had a correct diagnosis. Overall there was a 66.6% diagnostic accuracy rate. The earliest diagnosis of an extrauterine gestation sac was made at 6 weeks' gestation. Levels of plasma β-hCG 15 days after oocyte recovery ranged from 141 to 2710 IU/L, and serial assays over 20 days were frequently within the normal range for normal intrauterine pregnancies.

Outcome of pregnancies. The extrauterine gestation sac ruptured spontaneously in eight patients. Surgical excision was performed in 14 patients. Unilateral salpingectomy was performed in 10 patients, bilateral salpingectomy in two patients, and salpingo-oophorectomy in two patients. A local injection of potassium chloride in the fetal heart followed by aspiration of the gestation sac was performed in two patients under transvaginal ultrasonographic control. One extrauterine gestation sac resolved spontaneously.

At the time of the initial diagnosis, the viable intrauterine pregnancy was present in seven patients and twin intrauterine sacs were present in three others. One patient miscarried and the others progressed uneventfully with seven singleton deliveries, one twin, and one on-going pregnancy.

Case reports and series of heterotopic pregnancy after IVG–embryo transfer are presented in Table II.

Comment

There is a high incidence of combined heterotopic pregnancy after IVF, reaching 1% of all clinical pregnancies in our clinics between 1985 and 1989. Another recent series reported nine cases among 312 clinical IVF pregnancies (2.9%)[4] and a multicenter study that incorporated large IVF centers in the United Kingdom had 30 cases among 2234 clinical pregnancies (1.3%).[5] In comparison, the reported frequency of heterotopic pregnancies in spontaneous conception include 1 in 6778 from Israel,[2] 1 in 4112 from Belgium,[6] and 1 in 3889 from New York.[1] These estimates replace the oft-quoted theoretical incidence of the condition (e.g., 1 in 30,000[7]) determined on the basis of an assumed incidence of 0.37% ectopic pregnancy, and 1 in 15,600[8] on the basis of a 0.8% incidence of ectopic pregnancy. Therefore the risk of a combined pregnancy after IVF–embryo transfer seems to be 30 to 60 times higher than that after spontaneous conception.

The etiologic factors associated with ectopic and heterotopic pregnancies include previous pelvic inflammatory disease, pelvic surgery, associated uterine malformation, use of ovarian hyperstimulation drugs, and multiple embryo transfer. Pelvic inflammatory disease with attendant postinfection tubal damage is evidently a potential etiologic factor in approximately 45% of ectopic pregnancies, raising the incidence by tenfold and even up to 4% of all pregnancies when laparoscopy was used for diagnosis. A 1% incidence of combined intrauterine and extrauterine gestation was found among patients who took ovarian hyperstimulation drugs.[9] The incidence of heterotopic pregnancies would be expected to be high in IVF patients because many have pelvic inflammatory disease, stimulation is used routinely, and multiple embryo transfers are common. The difference in the incidence between our center (1%) and other units (2.9%) could be attributed to the high prevalence of tubal damage and microsurgery among their patients, as discussed by the authors.[4]

The procedure for embryo replacement could raise the chances of combined gestation (e.g., through the direct extrusion of embryos through the tubal ostium by hydrostatic forces).[10] A small volume (50 to 100 μl) of radiopaque dye placed high in the uterus during mock embryo transfer can move into the fallopian tube after a few minutes, then subsequently disappear, possibly by spilling into the peritoneal cavity.[11] Dye was totally or partially transferred into the fallopian tubes in 44% of patients who had a mock embryo transfer in the cycle before IVF. A higher rate of ectopic pregnancy arises if the transfer catheter passes beyond the midcavity, and a routine measurement of the uterine length by

Table I. Clinical presentation and ultrasonographic diagnosis

Patient no.	*Clinical*	*Ultrasonographic findings*				*Duration of pregnancy at time of diagnosis (wk)*	
		Type	*Gestational sac in utero*	*Fetal hearts in utero*	*Ectopic pregnancy*	*Intrauterine*	*Ectopic*
1	Abdominal pain	Abdominal	1	1	1	6	8
2	Abdominal pain	Abdominal	3	2	0	6	NO
3	Abdominal pain	Abdominal	1	0	0	5	NO
4	Abdominal pain	Abdominal	1	0	1	6	6
5	Abdominal pain	Abdominal	1	0	0	5	NO
6	Abdominal pain	Abdominal	1	0	1	7	7
7	Abdominal pain, bleeding	Abdominal	2	1	1	7	7
8	Abdominal pain, bleeding	Abdominal	1	0	0	7	NO
9	Abdominal pain, bleeding	Abdominal	1	0	1	8	8
10	Acute abdomen	Abdominal	1	0	0	5	NO
11	Acute abdomen	Abdominal	2	2	0	8	NO
12	Acute abdomen	Abdominal	1	1	0	6	NO
13	No symptoms	Abdominal + vaginal	1	1	1	7	7
14	No symptoms	Abdominal + vaginal	2	2	1	7	7
15	No symptoms	Abdominal + vaginal	1	1	1	7	7
16	No symptoms	Abdominal + vaginal	1	1	1	7	7
17	No symptoms	Abdominal + vaginal	1	1	1	7	7

Table II. Review of cases of heterotopic pregnancy after IVF–embryo transfer

Author	*Age (yr)*	*Infertility cause*	*Ovulation induction*	*No. of embryos*	*Volume*	*Fetus in utero*	*Fetus in tube*
Bearman et al.	26	Endometriosis	hMG	6	70	1	1
Dicker et al.	28	Mechanical	Pure FSH, hMG	4	30	1	1
Dimitry et al. (9 cases)	—	Mechanical	Bus, hMG	—	—	—	—
Lower et al.	31	Mechanical	CC, hMG	3	—	1	1
	29	Mechanical	hMG	4	—	1	1
Porter et al.	33	Mechanical	CC, hMG	6	10	1	2
Rizk et al. (17 cases)	30	Mechanical	CC, hMG	—	—	—	—
Sondheimer et al.	28	T-shape uterus	hMG	5	50	2	1
Snyder et al.	35	T-shape uterus	—	4	—	1	1
Yovitch et al.	34	Mechanical	CC, hMG	5	30	2	1

CC, Clomiphene citrate; *Bus,* buserelin.

ultrasonography has been recommended to place the embryos in midcavity.[12] Nevertheless, one case of ectopic tubal pregnancy arose despite carrying out embryo transfer under ultrasonographic guidance. Limitation of the volume of transfer medium to 10 to 20 μl may help to avoid ectopic implantation but our present series and the cases reported in Table II show that combined pregnancies can arise with volumes of <10 μl. The number of embryos replaced could also be important; 13 of our 15 patients received three or more, and in other cases the number varied between four and six.[10, 13-16]

Complete tubal occlusion was advocated by Steptoe and Edwards[17] 15 years ago to prevent ectopic pregnancy and this may have helped to reduce the incidence at Bourn Hall Clinic. Fifteen years later tubal occlusions are not performed at Bourn Hall because ultrasonographic oocyte retrieval has reduced the role of laparoscopic assessment before IVF. Bowel adhesions to the tube have occurred after diathermy, making oocyte recovery more difficult, and tubal occlusion will not prevent interstitial pregnancies.

There was a variable clinical presentation among our 17 patients—vaginal bleeding or abdominal pain recurred in nine patients and acute surgical abdominal emergencies in three patients. Five patients did not

have symptoms. The four most common signs and symptoms in heterotopic pregnancies at first examination were earlier reported to be abdominal pain, adnexal mass, peritoneal irritation, enlarged uterus, and the absence of vaginal bleeding in a patient suspected of having an ectopic pregnancy was suggestive of combined gestation rather than an ectopic pregnancy as in our patients, Nos. 1, 3, 4, 5, and 6.

A high index of suspicion of heterotopic pregnancy is required after IVF–embryo transfer. A preoperative diagnosis rate of only 10% has been reported, and a clear-cut ultrasonographic diagnosis before surgical intervention was made in only 14% of cases in a review of the world literature between 1979 and 1984.[18] Transvaginal is superior to transabdominal ultrasonography in the diagnosis of extrauterine pregnancies, and an early diagnosis before rupture offers the chance of therapy in selected cases by ultrasonography-guided aspiration procedures or laparoscopic surgery.

Conservative treatments by aspiration of the gestation sac under ultrasonographic control and an injection of methotrexate[19] or potassium chloride[20] have been reported earlier, and were successful in two patients who progressed to term in the present series, obviating the need for laparotomy or laparoscopy. We restricted aspiration under ultrasonographic control to those patients with fetal heart activity to avoid the misinterpretation of ovarian masses (hemorrhagic corpora lutea cysts) as ectopic pregnancies. Extreme care must be exercised to avoid inadvertent injection of potassium chloride into a maternal vessel, with careful follow-up because the ectopic pregnancy may rupture after treatment with potassium chloride.

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A risk-benefit analysis of elective bilateral oophorectomy: Effect of changes in compliance with estrogen therapy on outcome

Theodore Speroff, PhD,[a, b] Neal V. Dawson, MD,[a] Leon Speroff, MD,[c] and Richard J. Haber, MD[d]

Cleveland, Ohio, Portland, Oregon, and San Francisco, California

A bilateral oophorectomy at the time of elective hysterectomy is often performed to prevent ovarian cancer. The assumption that endogenous estrogen can be easily replaced with supplemental medication fosters the decision for routine oophorectomy. Published reports on the use of postmenopausal estrogen indicate that compliance is less than perfect. This fact could affect the overall outcome. Decision analysis techniques with Markov cohort modeling were used to evaluate the policy of elective bilateral oophorectomy. Results from studies judged methodologically sound were combined to determine values representing the influence of estrogen on coronary heart disease, breast cancer, and osteoporotic fracture. The decision tree also explicitly incorporated patient compliance. When compliance with estrogen therapy is assumed to be perfect, oophorectomy yields longer life expectancy than retaining the ovaries. When actual drug-taking behavior is considered, retaining the ovaries results in longer survival. This analysis highlights the importance of including the effects of patient compliance with treatment recommendations when the impact of a health policy decision such as prophylactic surgery is assessed. (Am J Obstet Gynecol 1991;164:165-74.)

Key words: Compliance, bilateral oophorectomy, estrogen therapy, clinical decision analysis

Having decided to undergo hysterectomy, a woman, together with her physician, is faced with the decision of whether to also undergo removal of the ovaries. The proportion of hysterectomies in which both ovaries were removed increased from 25% in 1965 to 41% in 1984.[1] Although elective bilateral salpingo-oophorectomy is performed on >50,000 perimenopausal women each year, wide geographic differences in surgical rates and patterns of physician practice suggest inconsistency in deciding on oophorectomy.[1-7]

A major reason for choosing oophorectomy is to avoid the risk of developing ovarian cancer, a disease known for its poor prognosis.[8-10] Implicit in the argument is the assumption that endogenous estrogen can be easily replaced with supplemental medication.[11, 12] In light of the literature on compliance, assuming perfect compliance with a regimen of estrogen is unwarranted.[13, 14] Less than perfect compliance suggests that the potential effects of estrogen deprivation should also be considered when oophorectomy is contemplated in perimenopausal women.[15-17]

The realization that compliance with estrogen therapy is imperfect and the emergence of new data on the competing benefits and risks of estrogen-related diseases prompted us to reappraise the practice of elective oophorectomy in perimenopausal women. Increased mortality caused by coronary heart disease or hip fracture resulting from estrogen deficiency might combine to offset the benefit derived from avoiding ovarian or breast cancer.[18] In this study a clinical decision analysis explores the implications of changes in both drug compliance and various competing disease risks on the policy of elective oophorectomy.[19]

From the Department of Epidemiology and Biostatistics[a] and the Division of General Internal Medicine, Department of Medicine,[b] Case Western Reserve University at MetroHealth Medical Center, Cleveland, the Department of Obstetrics and Gynecology, Oregon Health Sciences University,[c] and the Division of General Internal Medicine, San Francisco General Hospital, and the Department of Medicine, University of California, San Francisco.[d]

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Methods

The model. The population model consisted of white women undergoing elective hysterectomy. The decision for or against prophylactic bilateral salpingo-

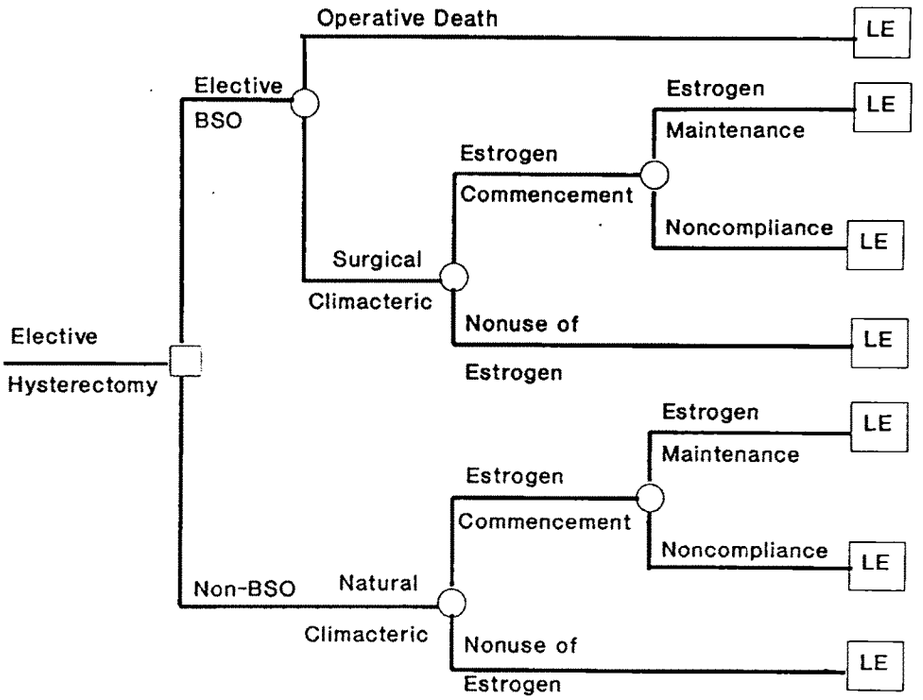


Fig. 1. Decision tree for question of whether to perform elective bilateral salpingo-oophorectomy *(BSO)* at time of hysterectomy. Life expectancy *(LE)* is simulated as a Markov cohort (see text for details).

oophorectomy at the time of hysterectomy divided the population into two groups (Fig. 1). Women who have their ovaries surgically removed submit themselves to a small increase in operative mortality as compared with those undergoing hysterectomy alone. Survivors experience surgical climacteric (i.e., sudden cessation of ovarian production of estrogen), which can be modified by a regimen of supplemental estrogen. Critical to restoring an estrogenic effect is whether exogenous estrogen is started (commencement compliance—estrogen prescribed and taken) and how long the estrogen is maintained (maintenance compliance). The course of events, modified by competing risks associated with estrogen status, is followed for the remaining life span of the patient.

Women who retained their ovaries constituted the alternative treatment strategy; the remaining life span of this nonoophorectomy group is also followed. Women in the nonoophorectomy group will have estrogen production until the time of natural climacteric (i.e., the natural decline in estrogen production by the ovary), which was fixed in this analysis at 50 years of age. A portion will then experience clinical climacteric and some or all will receive estrogen.[20, 21] For prophylactic purposes, some or all asymptomatic women could also receive estrogen. Preliminary evidence suggests that women who suffer clinical menopause have the same susceptibility to the outcomes of interest as those who experience few or only mild menopausal symptoms.[22, 23] The model therefore focuses only on the event of natural climacteric and subsequent use of supplemental estrogen. For either surgical or natural climacteric, the role of compliance (commencement and maintenance of estrogen) is considered. The course of events associated with natural climacteric and nonuse of estrogen is the baseline condition. Between the groups, only the risk for operative death, the risk for ovarian cancer, and the probabilities of estrogen-dependent events are altered.

The life-long course of events is simulated as a Markov cohort.[24, 25] The model allows probabilities of relevant events to differ with time and age. All women are assumed to be well initially and traverse through time until all members of the group die or reach the age of 100 years. Since the duration of temporary disability from a minor event is no longer than 1 year, the cycles were set at 1-year intervals. At each cycle the group traverses through the states of health in relation to the underlying transition probabilities.[24, 25]

During a 1-year cycle, a woman can remain well or experience one of the following adverse events (Table I): (1) spinal fracture, (2) wrist fracture, (3) hip fracture, (4) ovarian cancer, (5) breast cancer, (6) coronary heart disease, or (8) death from hip fracture, ovarian cancer,

Table I. Age-specific annual rates of fractures, ovarian cancer, breast cancer, and coronary heart disease

Age (yr)	Fractures			Ovarian cancer[30,31]	Breast cancer[32]	Coronary heart disease[33]
	Spine[26,27]	Wrist[28]	Hip[29]			
35-39	0	0.00098	0.000032	0.00012	0.00052	0.00063
40-44	0	0.00107	0.00022	0.00012	0.00104	0.00063
45-49	0	0.00179	0.00034	0.00033	0.00159	0.00232
50-54	0.005	0.00327	0.00050	0.00033	0.00172	0.00232
55-59	0.011	0.00455	0.00089	0.00056	0.00192	0.00787
60-64	0.011	0.00589	0.00153	0.00056	0.00226	0.00787
65-69	0.020	0.00495	0.00237	0.00058	0.00234	0.00985
70-74	0.023	0.00616	0.00530	0.00058	0.00260	0.00985
75-79	0.030	0.00476	0.01018	0.00053	0.00295	0.00987
80-84	0.030	0.00485	0.01731	0.00053	0.00301	0.00987
85-89	0.040	0.00633	0.03100	0.00038	0.00308	0.00987
90-100	0.040	0.00633	0.03700	0.00038	0.00308	0.00987

breast cancer, coronary heart disease, or other causes. The average number of cycles before death is equivalent to the group's life expectancy and the group with longer expected survival indicates the associated preferred choice of treatment. When morbidity of disease and quality-of-life issues are considered, the group with longer quality-adjusted life years indicates the preferred choice of treatment.

Mortality. The addition of oophorectomy to elective abdominal hysterectomy increases operative mortality. For hysterectomy with oophorectomy, operative mortality is 150% the operative mortality of elective abdominal hysterectomy without oophorectomy.[15] The age-specific (30 to 44 years) surgical mortality rate for low-risk abdominal hysterectomy is 2.8 per 10,000 operations.[34] Thus the incremented operative mortality rate for oophorectomy used in this study was 0.00014.

Mortality is associated with about 10% of the hip fractures that occur in self-sufficient, independently functioning women (Table II). Nonfatal outcomes of hip fracture include extreme loss of function necessitating long-term nursing home care, premanent disability that nevertheless allows independent living, or temporary morbidity followed by full recovery (Table II). A nonfatal hip fracture increases the risk of a subsequent hip fracture (relative risk = 1.6).[42] The outcome of a subsequent fracture depends on the morbidity resulting from the first occurrence, i.e., full recovery, disability, or nursing home care. The likelihood of adverse outcomes increasing with age is represented by an increase in annual incidence rate for fractures (Table I) and by the rate associated with a diminished state of health (Table II).

The annual mortality rates for cancer were modeled with the assumption that survival follows a simple declining exponential function.[43] A 37% 5-year survival rate for ovarian cancer[44] yields an estimated annual mortality rate of 0.1989, and a 75% 5-year survival rate for breast cancer[44] translates into an annual mortality rate of 0.0575. In the model, mortality as a result of cancer was calculated as incremental to the baseline age-specific mortality rate for women.

Table II. Probability of outcome associated with hip fracture

State	Outcome[35-41]			
	Full recovery	Disability	Nursing home care	Dead
Self-sufficient	0.50	0.20	0.20	0.10
Disability		0.44	0.41	0.15
Nursing home care			0.75	0.25

Note: Self-sufficient denotes all states of health from which a first incidence (Table I) of hip fracture can occur. Full recovery means a return to self-sufficiency after hip fracture and thus avoids fracture-related long-term morbidity; however, the relative risk for recurrence increases to 1.6.[42]

A first incidence of coronary heart disease (Table I) was proportioned among angina pectoris, nonfatal myocardial infarction, and fatal myocardial infarction (Table III). With the 10-year survival data reported by Hartunian et al.[33] and the declining exponential function, the annual mortality rate associated with angina was estimated at 0.021 per year. Again with the report by Hartunian et al.,[33] the annual rate for a myocardial infarction was estimated at 0.015 per year. In the model, mortality as a result of coronary heart disease was calculated as incremental to the baseline age-specific mortality rate for women. Given an initial nonfatal myocardial infarction, the subsequent annual risk for recurrent infarction was 0.1412 for women under 65 years of age and 0.2308 for women 65 and older.[33] The probability of death from the recurrent infarction was 0.2938 for women under 65 years of age and 0.6116 for women 65 and older.[33]

To model survival, age-specific mortality rates from life tables for white women were used.[45] The rates were

Table III. First-incidence coronary heart disease events: Proportions of angina pectoris, nonfatal myocardial infarction, and fatal myocardial infarction[33]

Age (yr)	*Angina pectoris*	*Nonfatal myocardial infarction*	*Fatal infarct*
35-44	0.57234	0.32219	0.10547
45-54	0.56897	0.32182	0.10921
55-64	0.64803	0.28394	0.06803
65-74	0.54924	0.26284	0.18792
75-100	0.54813	0.26377	0.18810

Table IV. Reported prevalence of postmenopausal estrogen use

	Commencement (%)*	*Maintenance† (%)*
Bilateral oophorectomy‡	31-89	13-71
Natural menopause§	21-60	5-34

*Commencement denotes proportion of women who receive prescriptions and start taking estrogen.

†Maintenance denotes 5-year compliance to a regimen of estrogen.

‡References 50, 51, 53, 55, 56, 61, and 64 to 67.

§References 50, 53, 55, 56, 64, and 66 to 69.

extended to age 100 by use of Gompertz age-function adjustments.[24, 46] To test our Markov model, the projected life expectancy with our model was compared with the life expectancy reported in the reference tables. With our model we calculated a life expectancy of 45.59 years for a 35-year old woman; the life expectancy reported in the reference tables is 45 years. All calculations were performed with SMLTREE, a decision analysis computer program written for use on an IBM personal computer.[47]

Rates and probabilities. Annual rates of incidence or mortality were converted to transition probabilities by the following formula[25]: Probability = 1 − Exp. (− Rate × Unit of time)

Competing risks associated with estrogen status. The baseline risks for coronary heart disease, breast cancer, and osteoporotic fracture were calculated from information previously published in the medical literature. The values extracted from the literature were estimates of relative risk of disease compared to the baseline condition: natural climacteric and nonuse of supplemental estrogen. The reported risks, weighted by their variance (precision of estimate), were combined to yield a baseline risk and 95% confidence interval.

With natural menopause the preponderance of the literature suggests a decreased risk for coronary heart disease associated with the use of postmenopausal estrogen.[48] The decrease in risk for coronary heart disease from three methodologically sound studies[49-51] was combined to yield a baseline risk of 0.45 (95% confidence interval = 0.33 to 0.61). The risk of osteoporotic fracture also decreases with use of postmenopausal estrogen.[52-54] For our baseline analysis we estimated risk for osteoporotic fracture at 0.54 (95% confidence interval: 0.42 to 0.71). For breast cancer the results from four studies[23, 55-57] indicate no association between breast cancer and postmenopausal estrogen use (risk = 0.93; 95% confidence interval: 0.78 to 1.12). Since the 95% confidence interval surrounds a risk of 1, we set baseline risk for breast cancer at 1.0.

For oophorectomy and no estrogen replacement, we estimated risk of coronary heart disease[49, 58-60] at 2.00 (95% confidence interval: 1.4 to 2.86) and risk of breast cancer[23, 55-57, 61] at 0.60 (95% confidence interval: 0.45 to 0.80). In addition, when no estrogen is taken, oophorectomy starts the progression toward postmenopausal osteoporosis at an earlier age.[62]

In relation to the decision model, estrogen replacement in oophorectomized women counters the elevated risk of coronary heart disease induced by oophorectomy (risk = 1.0) and, if maintained past the age of 50, provides protection (risk = 0.45) against coronary heart disease. Estrogen replacement in oophorectomized women delays the onset of osteoporosis and, if maintained past the age of 50, also provides protection (risk = 0.54) against osteoporotic fracture. However, estrogen replacement in oophorectomized women neutralizes the protection against breast cancer afforded by oophorectomy by returning the risk to a value of 1.0.[63]

Estimates of estrogen use and compliance. Commencement and maintenance constitute important components of estrogen compliance. Commencement denotes the proportion of women who receive prescriptions and start taking estrogen. Maintenance denotes the adherence to a regimen of estrogen over time. Prevalence of commencement and rate of maintenance were abstracted from publications on postmenopausal estrogen (Table IV). Most reports consider 5 years of taking estrogen as a long-term regimen.

For groups of women with oophorectomy, reported commencement of estrogen ranges from a low of 31% to a high of 89%, with 13% to 71% maintaining a long-term regimen. For naturally menopausal women, reported commencement of estrogen ranges from a low of 21% to a high of 60%, with 5% to 34% maintaining a long-term regimen. We assume that women who take estrogen for a long time are less likely to stop than women who have just begun taking it. Therefore maintenance was modeled by a simple declining exponential function with the annual probabilities of dropping out based on the reported 5-year estrogen compliance rate.[43]

Assessment. The full model included the decision

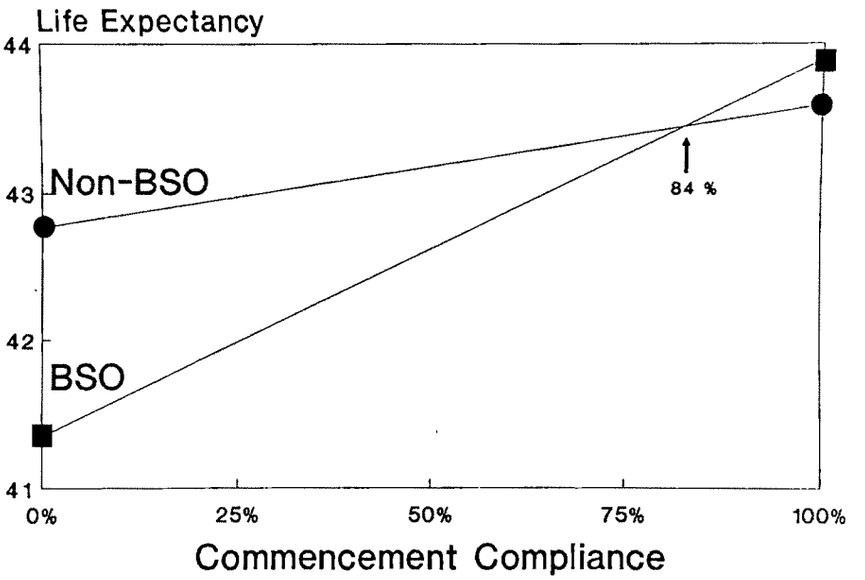


Fig. 2. Effect of oophorectomy *(BSO)* at age 35 and nonoophorectomy *(Non-BSO)* on life expectancy according to proportion of each cohort commencing estrogen therapy. Once estrogen is started, maintenance of estrogen use, as represented here, is perfect life-long compliance. *Filled squares* depict bilateral salpingo-oophorectomy group and *filled circles* depict the nonoophorectomy group.

tree with all competing risks and examined (1) the differences in life expectancy between the policy of oophorectomy versus nonoophorectomy, (2) the effects of variations in commencement and maintenance of estrogen therapy on life expectancy and policy selection, and (3) the effects of age at the time of operation. The ages considered were 35, 40, and 45 years.

Two components of compliance (commencement and maintenance) were varied from 0% (nonuse) to 100% (perfect compliance). This two-way sensitivity analysis on compliance produced a range of life expectancy for each policy (oophorectomy and nonoophorectomy). The range of life expectancy across various levels of compliance was then displayed graphically as a region of life expectancy associated with the chosen policy (oophorectomy or nonoophorectomy). Imposing the published limits of estrogen defined the regions of life expectancy associated with reported estrogen use. The bounded regions of life expectancy for oophorectomy and nonoophorectomy were then compared to identify the treatment with longer expected survival.

Sensitivity analyses were also conducted to verify the robustness of results to quantitative changes in competing risks associated with estrogen status. For the association between estrogen and coronary heart disease, the 95% confidence limits were used. For the association between estrogen and breast cancer, the risk was adjusted up to 2.00 for the nonoophorectomy group taking estrogen. In addition, a present-value analysis was calculated with the use of a discount rate of 5%.[19]

A sensitivity analysis on estrogen-disease relationships was performed by eliminating features from the full model. First, the influence of coronary heart disease was disregarded by setting all estrogen–coronary heart disease risks at 1.0. Second, the influence of breast cancer was also disregarded by setting all estrogen–breast cancer and estrogen–coronary heart disease risks at 1.0. The differences in life expectancy between policies, the effects of variable compliance, and the effects of age at time of operation were assessed in each restricted model.

Reduction in quality of life resulting from morbidity and disability associated with the various states of health was considered by analyzing quality-adjusted life years.[19] The results for quality-adjusted life years were qualitatively the same as the life expectancy results, indicating that considerations of life and death were of primary importance; therefore only the life expectancy results are presented.

Results

The results of Markov simulation on the full model for an operation at 35 years of age are presented in Fig. 2. The vertical axis depicts life expectancy and the left intercept depicts life expectancy associated with nonuse of estrogen (0% commencement). In the absence of estrogen replacement, retaining the ovaries (nonoophorectomy) leads to 1.4 more years of life expectancy than oophorectomy (42.8 years for nonoophorectomy vs 41.4 years for oophorectomy).

In Fig. 2, the proportion of women who start estrogen therapy is varied, while estrogen maintenance is

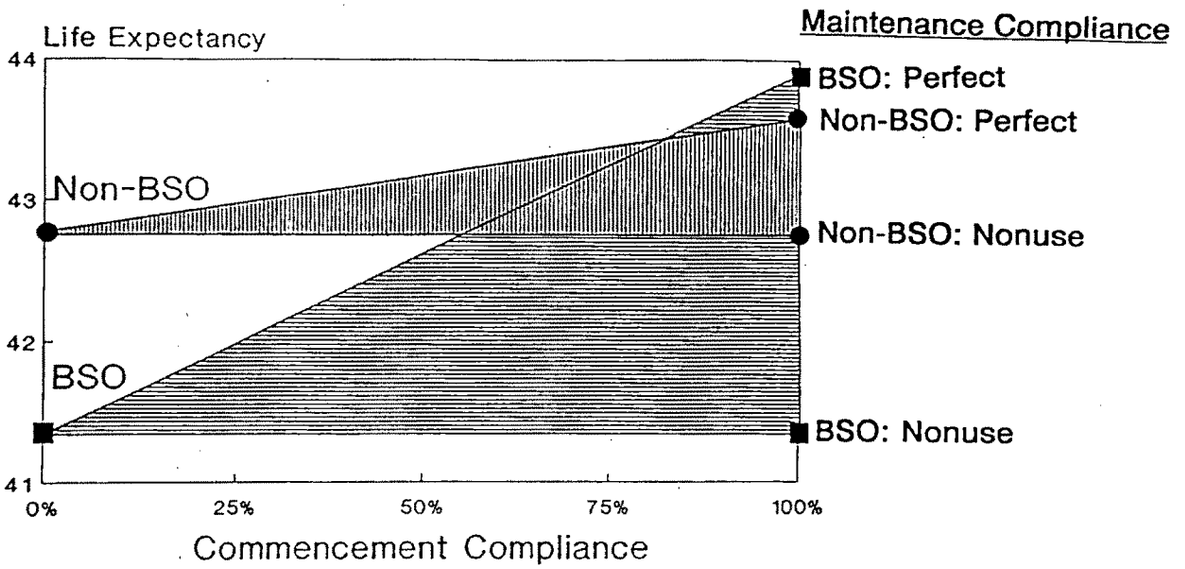


Fig. 3. Effect of oophorectomy *(BSO)* at age 35 and nonoophorectomy *(Non-BSO)* on life expectancy according to all variations in both commencement and maintenance of estrogen therapy. *Filled squares* depict the bilateral salpingo-oophorectomy group and *filled circles* depict nonoophorectomy group. The proportion of women commencing estrogen therapy varies from 0% (nonuse) to 100% along the horizontal axis. Maintenance varies from nonuse of estrogen to perfect life-long maintenance and is labeled along the right vertical axis. For nonoophorectomy the life expectancy resulting from any possible compliance combination (commencement and maintenance) falls within the *triangle* shaded by *vertical lines*. For oophorectomy the life expectancy for any given commencement and maintenance rate falls within the *triangle* shaded by *horizontal lines*.

held constant at 100% (perfect maintenance). Life expectancy increases as the proportion taking estrogen increases from a rate of 0% to 100% commencement. With perfect commencement and maintenance of a life-long regimen of estrogen, a shift in policy occurs. When compliance is perfect, oophorectomy is favored. Shown on the right side of Fig. 2, oophorectomy results in 0.3 more years of life expectancy than retaining the ovaries (43.9 years for oophorectomy vs 43.6 years for non-oophorectomy). The life expectancy lines for the two cohorts intersect at an 84% rate of estrogen commencement. When 84% of the oophorectomy group (at time of operation) and nonoophorectomy group (at time of natural menopause) start and maintain exogenous estrogen, life expectancy is the same for both groups.

In Fig. 3, both components of compliance are varied. Commencement of estrogen extends along the bottom horizontal axis and ranges from 0% to 100%. Maintenance of estrogen extends along the right vertical axis and ranges from nonuse (0%) to perfect compliance (100%) for each group. The borders formed by nonuse of estrogen, perfect life-long maintenance of estrogen, and the right-hand side of Fig. 3 form a triangle for each group (oophorectomy and nonoophorectomy) containing the life expectancies for all variations in drug compliance. For nonoophorectomy the region shaded by vertical lines indicates the life expectancy for every possible combination of estrogen commencement and estrogen maintenance; the region shaded by horizontal lines contains the life expectancy for every compliance possibility for oophorectomy.

Considering the observed lower and upper rates for estrogen commencement and maintenance (reported in Table IV), the shaded regions in Fig. 4 are formed. These shaded areas define a region of life expectancy associated with the published limits of estrogen use. The shaded life expectancy region for the oophorectomy group is below that of the nonoophorectomy group. These findings were replicated for the other ages tested. Thus, within the published limits of estrogen compliance, oophorectomy at the time of hysterectomy leads to a shorter life expectancy regardless of age at time of operation considering the age range of 35 to 45.

Among the modeled diseases, coronary heart disease is by far the most prevalent; it accounts for more than two thirds of the changes in life expectancy, dominates all other consequences, and is clearly the determining factor in the model. A sensitivity analysis on the association between estrogen and coronary heart disease was conducted with the confidence limits that lessen the impact of coronary heart disease (risk = 1.4 for oophorectomy and estrogen nonuse; risk = 0.61 for nonoophorectomy and estrogen replacement). Within the published limits of estrogen compliance, the life-expectancy region for the oophorectomy group re-

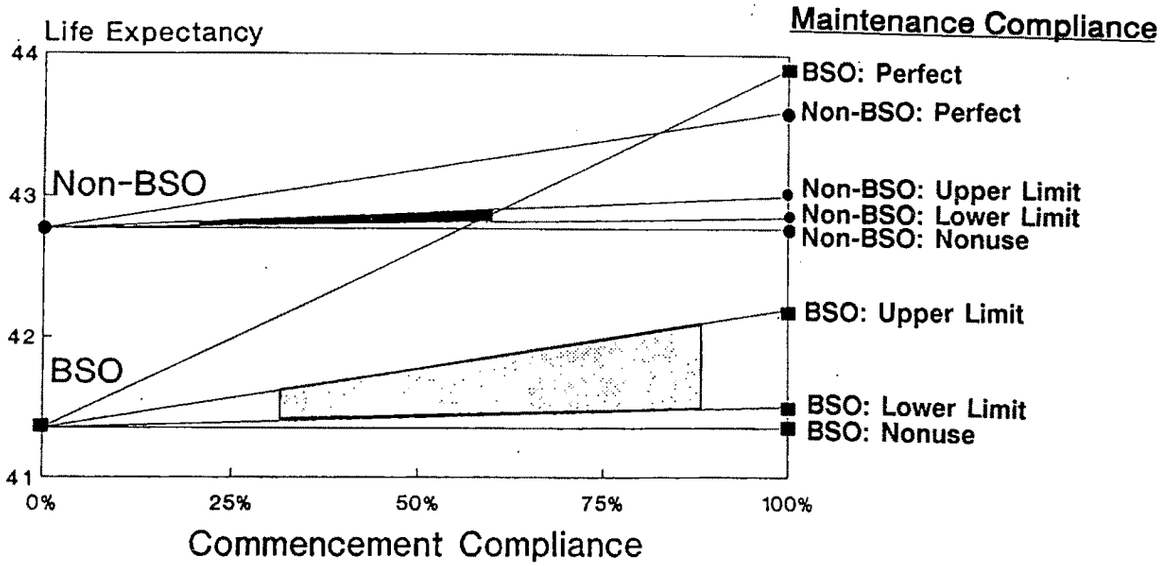


Fig. 4. Effect of oophorectomy *(BSO)* at age 35 and nonoophorectomy *(Non-BSO)* on life expectancy according to published limits of postmenopausal estrogen use. Lower and upper limits of estrogen maintenance reported in Table IV for nonoophorectomy and oophorectomy are labeled along the right vertical axis. The *shaded regions* indicate life expectancy within published limits for commencement and maintenance of estrogen. The *solid black* region indicates life expectancy for nonoophorectomy cohort and the *dot-shaded* region indicates life expectancy for oophorectomy cohort.

mained below that of the nonoophorectomy group at each age tested. Next, the risk of breast cancer associated with estrogen use was modified upward (risk = 2.0). The life-expectancy region for the oophorectomy group again remained below that of the nonoophorectomy group. Throughout sensitivity analysis, nonoophorectomy is the preferred option.

Values estimating the effect of estrogen on the risk of coronary heart disease and breast cancer were chosen from prominent, methodologically sound research. The literature on postmenopausal estrogen use and coronary heart disease, however, has been "controversial"[51, 70] and the literature on postmenopausal estrogen and breast cancer has been "mixed."[71, 72] Some physicians may prefer to disregard the effects of estrogen on these diseases until definitive results are published. For this reason, the decision analysis was repeated, first by disregarding the influence of coronary heart disease on the decision and second by disregarding the influence of both coronary heart disease and breast cancer on the decision.

When coronary heart disease is excluded from the model, breast cancer becomes a determining factor. In modeling, risk of breast cancer was not associated with use of estrogen in the nonoophorectomy group whereas protection against breast cancer was provided by oophorectomy in the oophorectomy group. Thus, regardless of estrogen compliance rates and regardless of age at the time of operation, oophorectomy leads to greater life expectancy than retaining the ovaries when the risk of coronary heart disease is excluded. In sensitivity analysis, adjusting the risk of breast cancer upward for the nonoophorectomy group (risk = 2.0) merely reinforces a preference for oophorectomy.

Excluding the effects of both breast cancer and coronary heart disease from the model, the trade-offs between hip fracture and ovarian cancer were examined. At age 45, the benefits of avoiding ovarian cancer more than offset the consequences of osteoporosis, making oophorectomy the treatment of choice regardless of estrogen use or nonuse. At age 40, oophorectomy remains the preferred approach. At age 35 and within the published limits of estrogen compliance, retaining the ovaries is preferable; a shift from nonoophorectomy to oophorectomy occurs during the late 30s.

A summary of the decisions leading to longer survival is presented in Table V. In addition, the procedure of 5% discounting was applied to each of the above analyses. Discounting favors oophorectomy by giving additional weight to the avoidance of ovarian cancer and breast cancer, which occur relatively earlier in life, and less weight to osteoporotic fracture, which tends to occur late in life. Even with discounting, the qualitative aspects of the findings remain unchanged.

Comment

Decision analysis has been widely applied to technology assessment and is used here to explore the health policy implications of estrogen treatment and elective oophorectomy at the time of hysterectomy.[73]

Table V. Health policy implications

Assessment	*Oophorectomy at time of hysterectomy*		
	35 yr	*40 yr*	*45 yr*
Full model	No	No	No
Coronary heart disease excluded	Yes	Yes	Yes
Coronary heart disease and breast cancer excluded	No	Yes	Yes

The results of the present analysis must be interpreted within the context it considers. For example, competing risks used in the analysis were derived from white women; other racial or ethnic groups may have different estrogen-disease relationships. Certain risk factors such as liver disease, thrombosis, or previous estrogen-sensitive tumor may contraindicate the use of supplemental estrogen; this analysis may not be applicable to women with such conditions. Brands, types, and dosage of postmenopausal estrogens were not differentiated, complex variations such as intermittent estrogen use were not considered, and other simplifying assumptions such as independence among events were made. When combined with estrogen, progestin may decrease risk of breast cancer and counteract the protective effect against coronary heart disease[74]; however, the data are inconclusive and the effects of low-dose progestin in combination with estrogen are just being examined.[75] Furthermore, the current model considered only women who had undergone a hysterectomy. In women without a uterus there is no concern about reducing the negative side effect of spontaneous bleeding that often accompanies estrogen treatment for clinical menopause. For these reasons progestin-estrogen combination medication was not considered in the present analysis.

Three decision analyses have evaluated the postmenopausal use of estrogen and another decision analysis has evaluated the effect of elective hysterectomy.[26, 76-78] Although the magnitude and pervasiveness of noncompliance is generally acknowledged in such evaluations, accounting for noncompliance often plays little or no role in assessment, decision making, policy setting, or individual patient care.[12, 79] Yet, when treatment involves medication, failure to comply can be the determining factor that hinders the goal of health care.[14, 80, 81]

A primary consideration at the time of elective hysterectomy has been the prevention of a future case of ovarian cancer, which is known for its poor prognosis.[9, 10, 15] When coronary heart disease and breast cancer were not considered in the analysis, the net results supported those who advocate routine prophylactic bilateral oophorectomy after the age of 40 years.[9-12, 82] When coronary heart disease alone is disregarded, survival is again greater with the choice of oophorectomy, regardless of estrogen use or nonuse. When coronary heart disease is factored into the model and perfect compliance to exogenous estrogen is assumed, a strategy of oophorectomy is again uniformly favored. The latter analysis, however, neglects the potential effects of variations in compliance with estrogen therapy. When the reported range of compliance with estrogen therapy is factored in the analysis, the preferred strategy changes.

Although consideration of patient behavior adds further uncertainty to the decision process, this added complexity is necessary to realistically compare one clinical strategy or program with another. In this investigation, elective oophorectomy at the time of hysterectomy is not recommended when actual physician prescribing and patient compliance behavior are considered in relation to all relevant estrogen-related diseases. Thus the ultimate decision on oophorectomy may well rely on the individual physician's and patient's assessment of ability to ensure long-term compliance to exogenous estrogen. This finding is in contrast to that based on perfect compliance and demonstrates that treatment based on what is efficacious may substantially differ from treatment based on what is effective.[83]

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Characterization of sodium channels in cultured human uterine smooth muscle cells

Roger C. Young, MD, PhD, and Lea Herndon-Smith
Charleston, South Carolina

Voltage-clamp studies using the whole-cell patch clamp technique were performed on single cells of cultured human uterine smooth muscle obtained from term pregnancies. The small size of the cells allowed time resolution of transient ionic currents as short as 3 msec. A large, voltage-activated inward current was identified as a sodium channel conductance by the following critiera: (1) Removal of sodium from the bath eliminated the current; (2) current was blocked by the sodium-channel-blocking agent tetrodotoxin; (3) current was observed in the absence of calcium. The sodium current was large (maximal inward current 7.2 $\mu A/cm^2$) and of short duration (decayed within 10 msec). The onset of activation of this current was −40 mV, with peak inward current at −10 mV. Steady-state voltage inactivation of this channel demonstrated half-maximal inactivation at −67 mV, indicating that this channel is largely inactivated at normal resting potentials. (Am J Obstet Gynecol 1991;164:175-81.)

Key words: Human uterine smooth muscle, electrophysiology, voltage-activated sodium channel

Uterine contractions are thought to be regulated largely by the free intracellular concentration of calcium.[1] Depolarization of the membrane is responsible for opening voltage-activated calcium channels,[2] which allows for the influx of calcium from the extracellular space. Any ion channel that modifies the active properties of the cell membrane potentially alters the capabilities of the cell to raise intracellular calcium concentrations.

Sodium conductance effects on action potentials were seen in rat uterine tissue after exposure to neurotoxins[3]; however, voltage clamp observation of a sodium current under physiologic ion concentrations has only recently been reported.[4] Sodium currents have not been previously reported in human uterine smooth muscle. We report here on large, rapidly decaying sodium currents observed in cultured human uterine myocytes under physiologic ion concentrations that are expressions of tetrodotoxin-sensitive, voltage-activated sodium channels.

From the Department of Obstetrics and Gynecology, Medical University of South Carolina.
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Reprint requests: Roger C. Young, MD, PhD, Department of Obstetrics and Gynecology, 171 Ashley Ave., Charleston, SC 29425.
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Material and methods

Cell culture. Small strips of pregnant human myometrium (0.5 × 0.5 × 2.0 cm) were excised from the upper margin of the uterine incision through the lower uterine segment at the time of cesarean delivery (Medical University of South Carolina Institutional Review Board Approval No. 3605). Serosa and endometrium were dissected free and discarded. The myometrium was minced into 1 mm cubes and washed in calcium-magnesium–free Hanks' solution (Sigma Chemical Co., St. Louis). The washed myometrial pieces were placed into a solution composed of calcium-magnesium–free Hanks' solution containing 0.2% (wt/vol) type III collagenase (Worthington Biochemical Corp., Freehold, N.J.), and maintained at 35° C for 30 to 120 minutes until free cells began to appear. The tissue and free cells were then centrifuged at 20 *g* for 5 minutes, the supernatant was discarded, and the pellet was washed in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, penicillin (100 U/ml, Sigma), and streptomycin (100 μg/ml, Sigma). Aliquots of the suspension were plated into 25ml culture flasks (Corning Medical and Scientific, Medfield, Mass.). The cells were kept in a humidified incubator gassed with 95% air plus 5% carbon dioxide at 37° C in the medium described above. On reaching confluence (usually 7 to 14 days), cells were freed from the flask with 1× trypsin, washed with culture media, and divided into three or four fresh 25ml culture flasks.

Table I. Composition of solutions

	Composition (mmol/L)				
Solution No.	Na^+	K^+	TEA^+	Ca^{++}	Mg^{++}
1	120	5	—	3	—
2	120	5	—	—	3
3	—	5	120	3	—
4	5	110	10	0.1	—

All solutions contain HEPES (5 mmol/L) at pH 7.2, adjusted with potassium hydroxide. Chloride is the counter ion for cations in the table. Solution 4 is the electrode-filling solution and also contains 1 mmol/L EGTA as a calcium buffer. Tetraethylammonium chloride (TEA^+) functions as a potassium-channel-blocking agent in solution 4 and as an inert cation for sodium substitution in solution 3.

Cells used for patch clamp studies were harvested between the third and sixth passage and had been maintained at confluence between 2 and 6 days.

Recording. The myometrial cells were freed from the bottom of the flask with 1× trypsin and 1.5 mmol/L ethylenediaminetetraacetic acid in calcium-magnesium–free Hanks' solution. The cells were washed in media once, followed by three washings with a solution composed of 120 mmol/L sodium chloride, 5 mmol/L potassium chloride, and 10 mmol/L HEPES, pH 7.2 ± 0.05. HEPES is 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid. An aliquot of this solution was placed into the recording chamber and the cells were allowed to settle and adhere to the glass bottom. The recording chamber was then flooded with an electrolyte bathing solution selected from Table I. Cells were observed with a Zeiss Axiovert 35 (Carl Zeiss, Inc., New York) inverted phase-contrast microscope.

Electrodes were pulled on a horizontal puller (Flaming-Brown, Sutter Instruments, San Rafael, Calif.) with borosilicate capillary tubes. Data reported here were obtained with seal resistances of at least 5 GΩ. Electrode resistances ranged from 4 to 7 MΩ when filled with electrolyte solution (solution 4, Table I). Tetraethylammonium chloride (10 mmol/L), a well-known potassium-channel-blocking agent,[5] was used in the electrode solution to reduce outward currents. Ethylene *bis*(oxyethylenenitrolo)tetraacetic acid (EGTA) was used as a calcium buffer to maintain free intracellular calcium concentrations in the nanomolar range.

Patch clamp recordings[6] were performed with the Axopatch 1-B amplifier (Axon Instruments, Foster City, Calif.). A 1 GΩ feedback resistor was used, and data were filtered through a four-pole Bessel filter set at 2 kHz cutoff. After gigaohm seal formation between the patch pipette and the cell membrane, intracellular access was accomplished with either moderate suction on the pipette or high-voltage pulses (1.6 V for several milliseconds).

Data were digitized with the Axolab 1100 data acquisition system and analyzed with p-CLAMP software (Axon Instruments) on a Compaq 286 computer. All recordings were made at 20° to 25° C. Electrode and stray capacitance was electrically compensated. Electrical compensation of the charging currents of the cell membrane capacitance was not used, as minor drift induced large current artifacts in the 1 to 10 msec time frame.

Voltage clamp data were obtained by applying voltage step or voltage ramp commands from the holding potential to test potentials while monitoring the membrane current. By standard convention, positive ions flowing into the cell constitute inward current, which is represented by a downward deflection of current. The magnitude and time course of membrane currents were stable over time (up to 1 hour). Five seconds between test pulses was allowed for the cell to reequilibrate.

Current-voltage curves were obtained by plotting observed currents versus test potentials. Membrane currents were measured from the holding level and, unless specifically noted, were not corrected for leak. Data for inactivation curves were obtained with the double pulse technique—a varying conditioning potential followed immediately by a constant test potential. These currents were corrected for leak before analysis. Curve-fitting of the inactivation curves was performed with a Marquardt nonlinear regression with the aid of STATGRAPHICS software (STSC, Inc., Rockville, Md.). The equation fit was the Hodgkin-Huxley formulation[7] for inactivation:

$$I_{in}/I_{in}(max) = [1 + \exp((V - V_{0.5})/k)]^{-1}$$

Results

Cultured human uterine myocytes exhibit large inward currents under voltage clamp conditions (Fig. 1, *A*). Outward current is reduced with the potassium-channel-blocking agent[5] tetraethylammonium chloride (10 mmol/L) in the electrode. Residual outward currents are apparent (here, 67 pA for the voltage pulse to 0 mV); however, they are small enough to allow clear observation of inward currents. In the presence of both sodium and calcium, two types of inward current can be identified (bath: solution 1). The larger current is a rapidly developing, rapidly decaying inward current with a current-voltage relationship showing the beginning of activation at −30 mV and peak inward current at −10 mV (Fig. 1, *B*). The smaller, more slowing decaying current activates at −40 mV. The peak inward current attributable to this conductance cannot be accurately determined under these conditions since the large fast transient dominates the tracing between −30 and +20 mV.

To determine the ion specificity of these conduct-

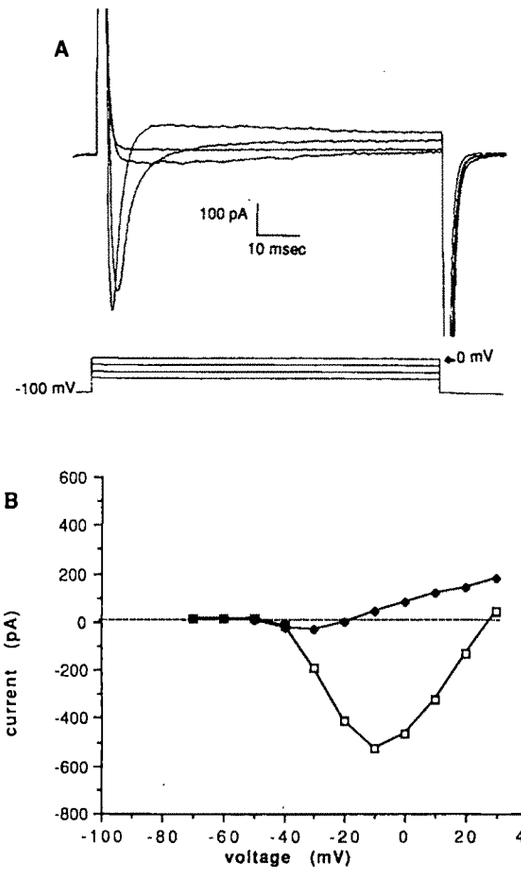


Fig. 1. A, Inward current recorded from cultured human myometrium with whole-cell voltage-clamp technique. Bathing solution contains 120 mmol/L sodium and 3 mmol/L calcium (solution 1). Lower tracing shows voltage paradigm. Holding potential is −100 mV. Four test pulses from holding to −30, −20, −10, and 0 mV are shown. **B**, Current-voltage relationship for peak inward current (□) and inward current that persisted longer than 20 msec (■).

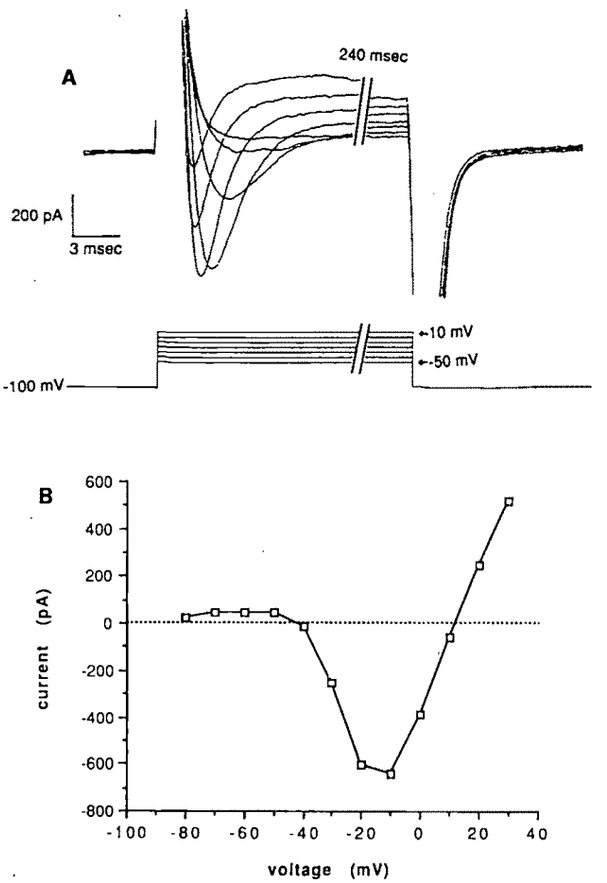


Fig. 2. A, Inward sodium currents observed with calcium-free bathing solution (solution 2). **B**, Current-voltage relationship for peak inward currents.

ances, ion substitution experiments were performed. Fig. 2, *A*, shows the current tracings obtained when magnesium is substituted for calcium (bath: solution 2). The large, fast transients persist, but the slower transients are not apparent. The current-voltage relationship of the peak inward current under these conditions (Fig. 2, *B*) is only slightly different than the current-voltage relationship obtained in the presence of calcium (Fig. 1, *B*). The onset of activation is −40 mV and the peak inward current is −10 mV in this calcium-free solution.

Fig. 3 is a comparison of current responses of the same cell under differing bathing compositions. From a holding potential of −100 mV, a test voltage of −10 mV was applied after exchange of bathing solution. Tracing a was obtained in the presence of both sodium and calcium (bath: solution 1) and is composed of a fast and a slow component. Tracing b was obtained after exchange of bathing solution 1 with a solution that did not contain calcium (bath: solution 2). Under these conditions, the fast transient persists, but the slow transient is much reduced. The 1 msec difference in time-to-peak current is a result of a small loss of time resolution that occurred during solution change. Exchange of the bathing solution with one that contains calcium but no sodium (bath: solution 3) yields tracing c, where the slow transient is observed but the fast transient is not. This experiment demonstrates the dependence of the fast transient on the presence of sodium, but not on the presence of calcium, and implies that the fast transient is the expression of a sodium channel.

Confirmation of the fast transient as a sodium channel current can be made by investigation of the sensitivity of the fast transient to tetrodotoxin, a specific sodium-channel-blocking agent.[8] The rapidly decaying inward current is lost as the tetrodotoxin concentration

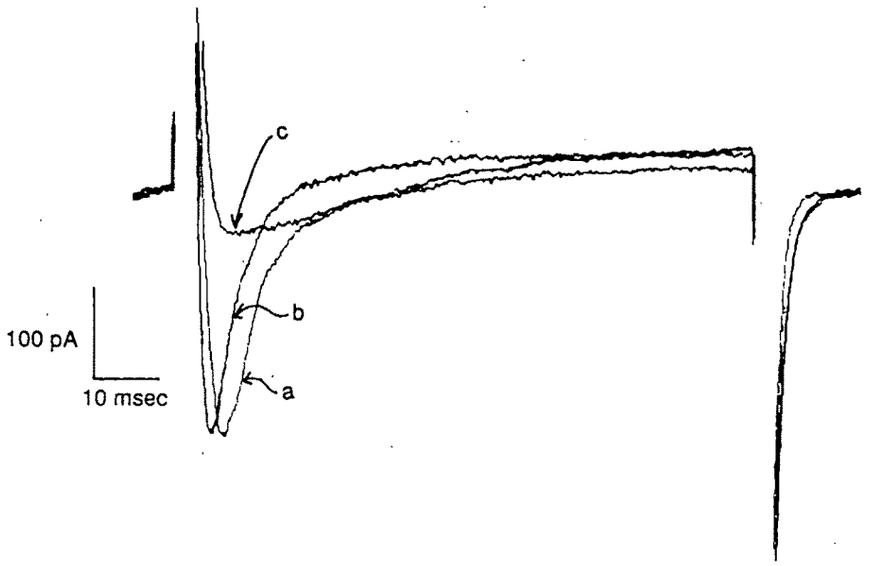


Fig. 3. Overlay of current responses resulting from identical test pulses from −100 to −10 mV on the same cell but with different bathing solutions. *a*, Solution 1 (sodium and calcium present). *b*, Solution 2 (sodium present, calcium absent). *c*, Solution 3 (sodium absent, calcium present).

is increased to 1 μmol/L and returns on rinsing the experimental chamber with tetrodotoxin-free bathing solution (Fig. 4).

The capacitance of the cell may be measured by applying a voltage ramp under voltage clamp conditions. The voltage ramp rises from −100 to +50 mV over 70 msec and then returns to a −100 mV holding potential. The step increase in current at the beginning of the ramp (Fig. 5, arrow) relates to the charging of the membrane capacitance by the relationship $\Delta i = Cm \times \Delta V/\Delta t$, where Cm is the membrane capacitance and $\Delta V/\Delta t$ is the magnitude of the voltage ramp applied (in this case 2.14 V/sec). The observed current step in Fig. 5 is 186 pA, which corresponds to a capacitance of 87 pF for this cell in solution 2. Assuming a 1 $\mu F/cm^2$ unit membrane capacitance, the cell membrane surface area is 8.7×10^{-7} cm^2. Changing bathing solutions from solutions 1 through 4 in Table I did not significantly affect the observed values of membrane capacitance. For a peak inward current of 630 pA (Fig. 2, *B*), the current density is calculated to be 7.2 $\mu A/cm^2$. The downward inflection of the current in response to the rising voltages (Fig. 5) yields a quasi-steady-state current-voltage relationship that is qualitatively similar to the peak inward current-voltage relationship obtained with the repetitive voltage pulse technique (Fig. 2, *B*).

The sensitivity of the sodium current to changes in the resting membrane potential can be demonstrated by a double-pulse experiment, where a conditioning voltage is applied for 250 msec, followed by a test pulse to 0 mV (Fig. 6, *A*). As the conditioning pulse becomes more depolarizing, the inward current induced by the test pulse is reduced. The resulting inactivation curve (Fig. 6, *B*) is well fit by the Hodgkin-Huxley formulation of a Boltzmann distribution curve yielding $V_{0.5} = -67$ mV and $k = 8.5$ mV. The bathing solution for this experiment was chosen to be as close to physiologic ion concentrations as possible. Specifically, calcium was not omitted. Since calcium currents are much smaller than the sodium currents, errors in the Boltzmann parameters caused by calcium currents are minimal.

Comment

This work describes a fast, voltage-activated sodium channel that is expressed in cultured human uterine myocytes. Ion substitution experiments demonstrate that sodium in the bathing solution is necessary for the expression of the rapidly decaying transient; however, calcium is not. Furthermore, the fast current is sensitive to micromolar concentrations of the sodium-channel-blocking agent tetrodotoxin.

For nerve tissue, tetrodotoxin typically ablates sodium channels at concentrations in the nanomolar range, where cardiac muscle,[8, 9] skeletal muscle,[8, 10] and rat uterine smooth muscle[4] sodium channels require 1 μmol/L for total block. Since the cultured human myometrial cells described here similarly require 1 μmol/L tetrodotoxin for block, they are tetrodotoxin

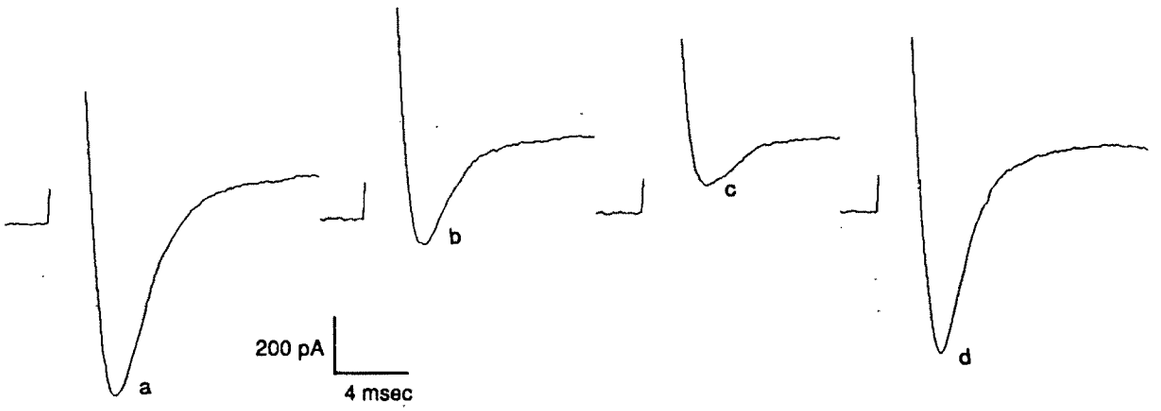


Fig. 4. Sodium current responses to increasing concentrations of tetrodotoxin, followed by washout of bath with tetrodotoxin-free solution (*a*, 0; *b*, 10^{-7} mol/L; *c*, 10^{-6} mol/L; *d*, washout). Bath: solution 2. Test pulses are from holding potential of −100 to −10 mV.

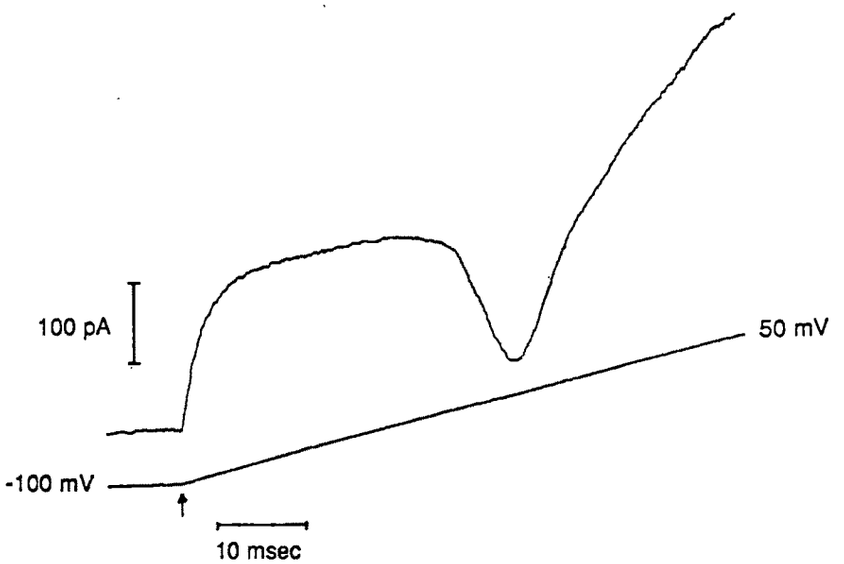


Fig. 5. Under voltage clamp conditions, a 70 msec voltage ramp is applied from holding potential (−100 mV) to +50 mV. *Arrow* marks onset of voltage ramp. Current needed to fully charge membrane capacitance is 186 pA, yielding a membrane capacitance of 87 pF (see text).

sensitive. Persistence of tetrodotoxin sensitivity of sodium channels in these cells, which have undergone at least three passages in culture, is in contrast to reports on cultured cardiac[9] and denervated skeletal[10] muscle, where tetrodotoxin sensitivity was rapidly lost.

Recently Ohya and Sperelakis[4] reported a sodium channel in myocytes freshly isolated from uteri of pregnant rats. They reported a maximum current density of 1.64 μA/cm² for the sodium channel in comparison to 7.2 μA/cm² seen here for cultured human myocytes. The relatively large value for peak sodium current is actually a lower limit, since the time resolution of our experiment is at best 3 msec, and the sodium current transient has a half-life of 2 to 4 msec. In other respects the similarity of the sodium channels is remarkable (Table II).

Inoue et al.,[11] in freshly isolated myometrium from human pregnancy, failed to observe a voltage-activated sodium current. The time resolution of the current tracings in the whole-cell voltage-clamp experiments is not reported and may not have been sufficient for resolution of the fast sodium current. However, in that report the resting membrane potential did vary with external sodium concentration.

The physiologic role of the sodium channel is not immediately apparent. The resting potential of freshly dispersed myometrial cells from human pregnancy has been measured with intracellular microelectrode tech-

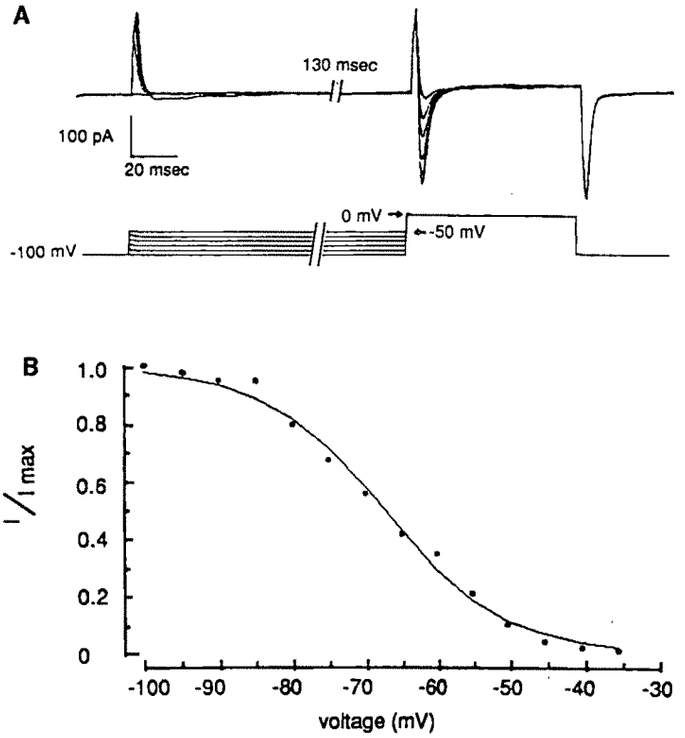


Fig. 6. A, Current responses to test pulse to 0 mV after various conditioning pulses (see Methods). Bath: solution 1. Conditioning pulse duration is 250 msec. **B**, Hodgkin-Huxley inactivation curve of peak inward current. Nonlinear regression of data *(solid line)* yields $V_{0.5} = -67$ mV, $k = 8.5$ mV.

Table II. Comparison of electrophysiologic parameters between sodium channels of rat[4] and human myometrium (this work)

	Activation (mV)		*Inactivation (mV)*	
	Onset	*Peak*	$V_{0.5}$	k
Rat	−40	0	−64	7.1
Human	−40	−10	−67	8.5

Data on rats were obtained on freshly dispersed uterine cells from pregnant rats, whereas data on human cells were obtained from cultured uterine myocytes. Both sets of activation data were obtained in calcium-free solutions with inactivation data obtained in physiologic calcium solution.

niques to be around −50 mV, although there was a large amount of cell-to-cell variability.[11, 12] At this potential and under physiologic ion concentrations (bath: solution 1), the sodium channels are significantly inactivated (Fig. 6, *B*). More important, the inactivation of the sodium channel required a conditioning pulse duration of only 250 msec (Fig. 6, *A*), which was specifically chosen to be of shorter duration than slow-wave membrane potential changes. Thus, as a slow wave depolarizes the cell to the potential at which calcium channels begin to open (approximately −40 mV),[11] the sodium channels will be largely inactivated and will not likely contribute to the rising spike of the action potential. This reasoning is in agreement with our observation that substitution of half of the extracellular sodium with tetraethylammonium chloride fails to reduce the maximal rise of the action potential spike in freshly dispersed human uterine myocytes.[13] Thus sodium currents cannot be demonstrated to significantly contribute to the rising phase of the action potential in human myocytes.

Trypsin was used to release the cells from the culture flasks and may have modified the activation or inactivation properties of the channel.[14] The close agreement of the electrical properties of the sodium channels of these human cells with those found for the sodium channels of rat uterine cells isolated with the use of collagenase[4] argues against significant channel modification by enzymes.

Whenever possible, conditions were chosen to minimize deviations from physiologic conditions. For example, suppression of outward currents was found to be adequate with the use of only 10 mmol/L tetraethylammonium chloride in the electrode solution, although harsher conditions such as total replacement of intracellular potassium are more effective. The use of

cesium (a potent potassium-channel-blocking agent) was attempted but abandoned because of cell destruction after approximately 10 minutes of experimentation.

We chose a 250 msec duration for the conditioning pulse in the double-pulse sodium-channel inactivation experiment rather than the usual duration of several seconds[14] to determine that inactivation occurs on a time scale that is shorter than that of uterine slow-wave depolarization. Additionally, 3 mmol/L calcium was used in the sodium-channel inactivation experiment to prevent adverse effects of nonphysiologic calcium solutions.[15]

Cultured human uterine cells seem to be an appropriate model system for the study of ion channels of human myometrium. The improved time resolution inherent to the smaller cell size allows quantitative characterization of the fast sodium channel by means of single-electrode techniques.

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Cocaine directly augments the α-adrenergic contractile response of the pregnant rabbit uterus

William W. Hurd, MD,[a, b] Patricia A. Robertson, MD,[a] R. Kirk Riemer, PhD,[a, b] Alan Goldfien, MD,[a, b] and James M. Roberts, MD[a, b]

San Francisco, California

Cocaine use in pregnancy is associated with a premature labor rate as high as 50%, but little is known about its effect on uterine contractility. To determine whether cocaine directly augments pregnant uterus contractility, uterine strips from 27-day pregnant New Zealand White rabbits (term, 31 days) were exposed to cocaine alone (30 μmol/L) or cocaine plus epinephrine (10^{-9} to 10^{-5} mol/L) or oxytocin (10^{-10} to 10^{-6} mol/L). Cocaine alone produced no contractions, but increased the epinephrine sensitivity by 51% and the maximal response by 33%. When β-adrenoceptors were blocked with DL-propranolol (2 μmol/L), the contractile response to epinephrine was increased, and cocaine's effect was blocked. In the presence of the stereoisomer D-propranolol (2 μmol/L) with no β-adrenergic antagonist activity, the contractile response to epinephrine was unchanged, but the effect of cocaine was still blocked. We conclude that cocaine directly augments the α-adrenergic contractile response of the pregnant rabbit uterus by a mechanism that is blocked by the non-β-adrenergic effects of propranolol. (AM J OBSTET GYNECOL 1991;164:182-7.)

Key words: Cocaine, pregnancy, propranolol, premature labor, drug abuse, catecholamines, adrenergic receptors

Drug abuse has become a major health concern in the United States, involving an estimated 15% of women of childbearing age.[1] The use of cocaine has been found to be especially deleterious during pregnancy, where it is associated with, among other things, a premature labor rate of 17% to 50%.[2-4] Unfortunately, little is known about how cocaine might affect uterine contractility and thus predispose pregnant women to premature labor.

Although cocaine may augment uterine contractility by a systemic effect such as increased circulating catecholamines,[5] direct effects of cocaine on the uterus may be at least as important. Cocaine is known to augment the contractile response to several agonists, most notably catecholamines, in many different types of muscles.[6-10] In addition, cocaine alone has been reported to directly induce contractions in nonpregnant uteri of rabbits and rats,[11, 12] although other investigators have found no contractile effect of cocaine alone.[13]

From the Department of Obstetrics, Gynecology and Reproductive Sciences[a] and the Cardiovascular Research Institute,[b] University of California, San Francisco.
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Reprint requests: William W. Hurd, MD, Department of Obstetrics and Gynecology, University of Michigan Medical Center, 1500 E. Medical Center Dr., Ann Arbor, MI 48109-0718.
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The best characterized mechanism by which cocaine augments contractile response is inhibition of catecholamine uptake by sympathetic nerve endings, thus increasing the local concentration of catecholamines.[14] However, because the pregnant uterus has few functioning adrenergic nerve endings after midpregnancy,[15, 16] inhibition of neuronal uptake is unlikely to play a significant role in the association of cocaine with premature labor. Cocaine has also been shown to potentiate the α-adrenergic contractile response of tissue known to be free of sympathetic innervation such as human umbilical artery.[6] This could be a result of either a selective effect on α-adrenoceptors or a general effect on smooth muscle that increase contractility.[6]

This study was designed to answer the following two questions. First, does cocaine, alone or in conjunction with other agonists, augment the contractility of the pregnant rabbit uterus. And second, is cocaine's effect selective for the α-adrenergic contractile system rather than a more general effect on uterine contractility. To address these questions, we studied the effect of cocaine on the in vitro contractile response of the pregnant rabbit uterus both alone and in the presence of epinephrine, oxytocin, or potassium chloride. The epinephrine response was further characterized by the addition of the β-adrenoceptor antagonist DL-propranolol and the stereoisomer D-propranolol, which does not block β-adrenoceptors.[17]

The results of this study indicate that in our system, cocaine alone has no direct contractile effect on the pregnant rabbit uterus but augments the α-adrenergic

contractile response of the uterus by a selective mechanism that is blocked by the non-β-adrenergic effects of propranolol.

Material and methods

Animals and tissue preparation. Timed-pregnant New Zealand White rabbits (White Hare Rabbitry, Stark City, Mo.) weighing 2.7 to 3.2 kg were cared for under the guidelines of the University Animal Care Facility. At 27 days' gestation (term, 31 days) the rabbits were given lethal intravenous injections of pentobarbital (50 mg/kg) and portions of the uteri were quickly removed and placed in Krebs solution.

Drugs. The drugs used were epinephrine and DL-propranolol (Sigma Chemical Co., St. Louis), oxytocin (Calbiodem Co., La Jolla, Calif.), D-propranolol (Ayerst Laboratories, New York, N.Y.), and cocaine hydrochloride (pharmaceutical grade). All drugs were dissolved in 1 mmol/L hydrochloric acid.

Evaluation of contractile activity. Contractile activity was determined as previously described.[13] Briefly, portions of the uteri were trimmed of excess fat and connective tissue and cut into uniform longitudinal 12 mm × 3 mm strips. These strips were then suspended in 20 ml tissue baths filled with Krebs solution (composition in millimoles per liter: sodium chloride, 118.3; potassium chloride, 4.7; calcium chloride, 2.5; magnesium sulfate, 1.18; potassium hypophosphate, 1.17; sodium bicarbonate, 25.0; and glucose, 11.1) aerated with 95% oxygen and 5% carbon dioxide (pH 7.4) at 37° C. The strips were allowed to equilibrate at 2 *g* tension for 1 hour, during which time the Krebs solution was replaced every 30 minutes. Isometric contractile activity was measured with force transducers (Grass Instruments, Quincy, Mass.). Data were digitized at a rate of 60 points per minute and recorded on a magnetic disk by microcomputer (Apple Corporation, Cupertino, Calif.). Replicate strips from each rabbit were averaged to obtain a mean response for each experimental condition. Contractile force was quantified by integrating the area under the tension versus time curve for 90 seconds after addition of each agonist dose.

Protocol. The effect of cocaine alone was determined by exposure of uterine strips to cocaine hydrochloride (30 μmol/L) and recording contractile activity both immediately and after 30 minutes of continuous exposure. To determine the effect of cocaine on the contractile response to other agonists, immediately after the addition of cocaine hydrochloride (30 μmol/L), the strips were exposed to increasing concentrations of epinephrine (10^{-9} to 10^{-5} mol/L) or oxytocin (10^{-10} to 10^{-6} mol/L) every 90 seconds to generate cumulative concentration-response curves. The effect of cocaine on receptor-independent contractility was evaluated by exposure of fresh strips to a single depolarizing dose of potassium chloride (70 mmol/L) immediately after the addition of cocaine hydrochloride (30 μmol/L). To determine whether the effect of cocaine on the adrenergic contractile response was a result of an interaction with the β-adrenergic contractile mechanism, β-adrenoceptors were blocked by pretreatment for 30 minutes with DL-propranolol (2 μmol/L) before exposure to increasing concentrations of epinephrine (10^{-9} to 10^{-5} mol/L). To determine whether propranolol's ability to block the effects of cocaine was a result of β-adrenergic antagonism or to other effects of propranolol, DL-propranolol was replaced with D-propranolol, the stereoisomer of propranolol with no β-adrenergic blocking ability,[17] and the experiment was repeated.

Data analysis. Cumulative concentration-response curves were compared in terms of sensitivity (EC_{50}) and maximal contractile response (E_{max}). EC_{50}, defined as the concentration of agonist necessary to elicit a response equal to 50% of the maximal response, was calculated graphically for each uterine strip and the results were averaged for each rabbit. E_{max}, defined as the greatest integrated area under the force versus time curve developed during a concentration-response experiment, was expressed as a percentage of the maximal response to epinephrine alone. EC_{50} and E_{max} values were compared by a two-way analysis of variance followed by Duncan's multiple range test. A value of $p < 0.05$ was considered significant. Data are presented as the mean ± the SEM.

Results

Cocaine alone did not produce contractions in uterine strips from 27-day pregnant rabbits either acutely or at any time during a 30-minute observation period (Table I). However, cocaine did decrease the EC_{50} to epinephrine by 51% (Figs. 1 and 2, *A*) and increased the E_{max} to epinephrine by 33% (Figs. 1 and 2, *B*). Cocaine had no effect on the E_{max} to oxytocin or potassium chloride depolarization (Table I). Nor did cocaine have an effect on the EC_{50} to oxytocin (control, 4.9 ± 1.8 nmol/L; with cocaine, 6.9 ± 3.6 nmol/L).

When β-adrenoceptors were blocked with DL-propranolol (2 μmol/L) before epinephrine stimulation, the EC_{50} was decreased by 31% (Fig. 2, *A*), and E_{max} was increased by 43% (Fig. 2, *B*). The addition of cocaine prevented the increase in both sensitivity and maximal response. When DL-propranolol was replaced with the stereoisomer D-propranolol (2 μmol/L), the epinephrine response was not altered (Fig. 2). However, cocaine's ability to augment epinephrine sensitivity and maximal response was still prevented.

Comment

The results of this study indicate that whereas cocaine alone has no direct contractile effect on the pregnant rabbit uterus, it does augment the α-adrenergic con-

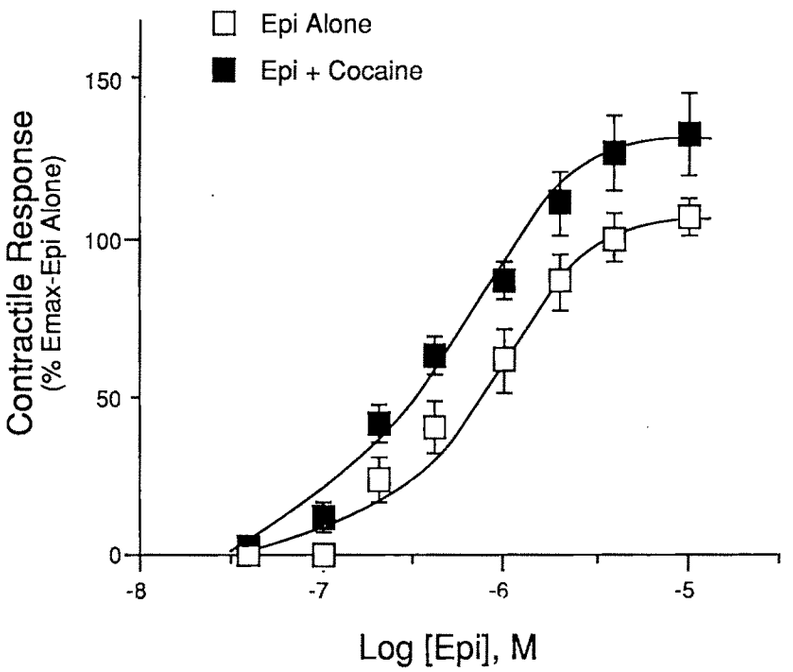


Fig. 1. Concentration-response curve for epinephrine with or without cocaine. Duplicate uterine strips from 27-day pregnant rabbits were exposed to increasing concentrations of epinephrine (10^{-9} to 10^{-6} mol/L) either alone *(Epi Alone)* or in presence of 30 μmol/L cocaine hydrochloride *(Epi + Cocaine)*. Data are expressed as percentage of maximal response to epinephrine alone (mean ± SE); $n = 8$ rabbits.

Table I. Effect of cocaine on the uterine contractile response alone, with potassium chloride and with oxytocin

Agonist	*Concentration (μmol/L)*	*Time of evaluation*	*No.*	*E_{max} (% epinephrine alone)*	
				Control	*Plus cocaine (30 μmol/L)*
Cocaine alone	30	Immediately	8	0 ± 2	0 ± 1
Cocaine alone	30	After 30 min	4	0 ± 10	7 ± 7
Potassium chloride	70	Immediately	8	83 ± 9	88 ± 5
Oxytocin	10	Immediately	7	88 ± 13	77 ± 9

Data are expressed as mean ± SE.

tractile response of pregnant rabbit uterus by a selective mechanism that is blocked by the non-β-adrenergic blocking effects of propranolol. When both α- and β-adrenoceptors are stimulated by epinephrine, cocaine increases the E_{max} by >50% and increases the sensitivity (i.e., decreases the EC_{50}) by >30% (Figs. 1 and 2). This effect seems to be selective for the adrenergic system because cocaine had no effect on the contractile response to either oxytocin or potassium chloride depolarization (Table I).

It was somewhat surprising to find that cocaine alone has no direct contractile effect on the pregnant rabbit uterus inasmuch as cocaine has been reported to induce contractility in the nonpregnant estrogen-treated rabbit uterus with an EC_{50} of 25 μmol/L.[11] However, in previous studies conducted in our laboratory, cocaine hydrochloride (20 μmol/L), used to prevent neuronal uptake of catecholamines by uterine strips from nonpregnant estrogen-treated rabbits, did not induce contractility.[13] Perhaps this lack of response to cocaine alone is a result of subtle differences in our experimental conditions.

Cocaine's ability to augment the adrenergic contractile response was further characterized by the addition of DL-propranolol or D-propranolol. In an effort to determine whether cocaine's effect was on the α-adrenergic contractile system, the tissue was incubated with the β-adrenergic antagonist, DL-propranolol (2

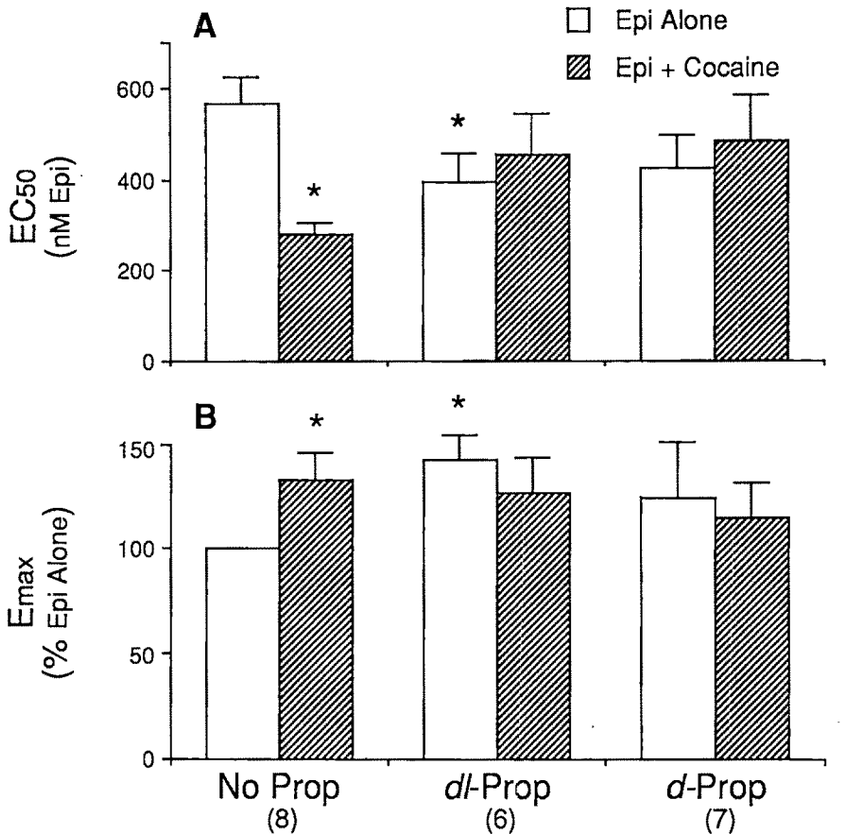


Fig. 2. A, Contractile sensitivity (EC 50) and **B,** maximal contractile response (E_{max}) for epinephrine. Duplicate uterine strips from 27-day pregnant rabbits were exposed to increasing concentrations of epinephrine (10^{-9} to 10^{-6} mol/L) either alone *(Epi Alone)* or in presence of 30 μmol/L cocaine hydrochloride *(Epi + Cocaine)*. Before epinephrine exposure, strips were equilibrated for 1 hour alone *(No Prop)*, in presence of 2 μmol/L DL-propranolol *(DL-Prop)*, or in presence of 2 μmol D-propranolol *(D-Prop)*. Data are average EC 50 (nmol/L epinephrine) determined graphically **(A)** or the average E_{max} expressed as percentage of maximal response to epinephrine alone **(B)** from concentration-response curves including those depicted in Fig. 1. Data are expressed as mean ± SE with number of rabbits indicated in parentheses. (*$p < 0.05$ versus epinephrine alone, no propranolol.)

μmol/L). The ability of DL-propranolol to increase both the sensitivity and the maximal response (Fig. 2) was expected because β-adrenoceptor stimulation results in uterine relaxation. But the loss of cocaine's ability to increase either sensitivity or maximal response was unexpected. On first evaluation, this seemed to indicate that cocaine may work by interfering with β-adrenergically mediated relaxation. To further clarify this observation, DL-propranolol was replaced with the stereoisomer D-propranolol (2 μmol/L), which does not block β-adrenoceptors.[17] As was expected, the epinephrine response was not altered. But in addition, cocaine's ability to augment epinephrine sensitivity and maximal response was also prevented (Fig. 2). This finding suggests that cocaine augments contractility by an effect on the α-adrenergic system, which can be blocked by the non-β-adrenergic effects of propranolol. This effect of cocaine and the ability of D-propranolol to inhibit it could be further characterized by the use of a pure α-adrenergic agonist (e.g., methoxamine hydrochloride) and this approach will be used in future studies.

Propranolol has been well established to have membrane-stabilizing properties in cardiac muscle that does not involve the adrenergic system.[17] This quinidine-like local anesthetic property of propranolol results in both decreased membrane responsiveness and decreased action-potential amplitude as well as decreased excitability and action potential velocity.[18] Because D-propranolol retains membrane-stabilizing ability but has no β-adrenoceptor blocking activity,[17] membrane stabilization may be the way in which propranolol blocks cocaine's ability to augment the α-adrenergic contractile effect.

The ability of propranolol to block cocaine's aug-

mentation of the α-adrenergic response may explain both experimental and clinical observations made when cocaine was evaluated in the presence of propranolol. First, cocaine has been found to augment adrenergic contractility in several denervated tissues if propranolol is not used.[6-8] However, when propranolol has been used to block β-adrenoceptors, no effect of cocaine is seen.[9, 10] Of more interest is the finding that propranolol has been found to decrease the mortality associated with cocaine use in several species including human beings,[19, 20] presumably by prevention of hyperstimulation of β-adrenoceptors in the heart and thus avoidance of tachyarrhythmias. Perhaps propranolol's non-β-adrenergic effects account for some of its ability to block cocaine effects in vivo.

Although this study indicates that cocaine augments the contractile response of the pregnant uterus by a direct effect, cocaine could predispose the pregnant uterus to premature labor by indirect mechanisms. Cocaine is best known for its complex actions on the central nervous system where, among other things, cocaine increases catecholamine and acetylcholine levels.[21] Because both catecholamines and acetylcholine stimulate the release of oxytocin,[22] cocaine may increase uterine contractility by increasing the level of circulating oxytocin. Unfortunately, no information is available on the effects of cocaine on oxytocin release.

Alternatively, it has been postulated that cocaine might predispose the pregnant uterus to premature labor because of its known ability to increase circulating levels of epinephrine and norepinephrine.[1, 5] However, available evidence suggests that increased levels of circulating catecholamines may actually inhibit contractility of the pregnant uterus. When epinephrine is administered during human labor, coordinate uterine activity is diminished.[23] Conversely, when norepinephrine is administered, only incoordinate uterine activity is increased.[23] Furthermore, when epinephrine and norepinephrine are increased during labor as a result of maternal anxiety, the length of labor is actually increased, suggesting an inhibitory effect of increased catecholamines on uterine contractility in vivo, probably mediated through β-adrenoceptors.[24] However, the direct effects of cocaine shown in our study may convert the net effect to an increase in uterine contractility when circulating levels of epinephrine and norepinephrine are increased by cocaine use.

The degree to which the results of this study may apply to the use of cocaine by pregnant women is uncertain because of possible interspecies differences and because of the cocaine concentration studied. Although adrenoceptor function is believed to be similar in the rabbit and the human uterus,[13] no previous study of which we are aware has addressed the effect of cocaine on the pregnant uterus of either species. Furthermore, the concentration of cocaine used in this study (30 μmol/L) is approximately 10 times the serum concentration associated with smoking alkaloidal "crack" cocaine[14] and thus may not reflect the condition seen in human cocaine users. However, the results do suggest that cocaine may selectively augment the contractile response of the pregnant uterus to α-adrenergic stimulation.

These data also have important implications for experimental design. Studies of adrenergic responses of smooth muscle in vitro are characteristically performed in the presence of cocaine to block neuronal uptake.[13] Conversely, the effects of cocaine are often studied in the presence of propranolol to selectively evaluate the α-adrenergic response.[9, 10] This study shows that not only can the effects of propranolol be masked by cocaine in vitro but also that the effects of cocaine can be masked by propranolol. (Fig. 2).

In summary, this study showed that cocaine alone has no direct contractile effect on the pregnant rabbit uterus, but that it augments the α-adrenergic contractile response by a selective mechanism that can be blocked by the membrane-stabilizing effects of propranolol. This information raises the possibility that the increased rate of premature labor associated with cocaine use in pregnancy may be related to direct effects of cocaine on the pregnant uterus.

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Spontaneous and steroid-induced recurrence of endometriosis after suppression by a gonadotropin-releasing hormone antagonist in the rat

Kathy L. Sharpe, PhD, Maria C. Bertero, MD, Kenneth N. Muse, MD, and Michael W. Vernon, PhD

Lexington, Kentucky

Recurrent endometriosis in women is difficult to study because of the ethical consideration of performing repeated surgeries. Previously in the rat model we described therapeutic regression of endometriosis with the gonadotropin-releasing hormone antagonist antide. Presently we report the spontaneous and steroid-induced recurrence of endometriosis after withdrawal from antide therapy. Rats with endometriosis received antide or vehicle on days 0 (proestrus), 3, 6, and 9 and were killed on days 0, 6, 12, 18, 24, 30, and 42 (n = 4 antide-treated and 4 vehicle-treated rats killed per day). Additional antide-treated rats (n = 4 per treatment) received estrogen, progesterone, both estrogen and progesterone, cholesterol, and no steroid on day 9 and were killed on day 12. Antide significantly suppressed endometriotic implant size on days 12, 18, and 24. However, implant size spontaneously returned to pretreatment values by day 30. Administration of steroids on day 9 elicited regrowth of antide-suppressed endometriosis (estrogen plus progesterone greater than estrogen, progesterone, or cholesterol greater than no steroid) by day 12. This resilience of endometriosis offers an explanation for treatment failure and recurrence of the disease in women. (AM J OBSTET GYNECOL 1991;164:187-94.)

Key words: Endometriosis, gonadotropin-releasing hormone, antide, rat endometriosis model

From the Kentucky Center for Reproductive Medicine, Department of Obstetrics and Gynecology, University of Kentucky.
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Reprint requests: Michael W. Vernon, PhD, Department of Obstetrics and Gynecology, University of Kentucky Medical Center, 800 Rose St., Lexington, KY 40536.
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Many drug therapies have been used in the treatment of endometriosis with varying degrees of success. Estradiol and progesterone preparations were initially thought to cause regression of the disease by inducing a state of pseudopregnancy.[1] Danazol interacts with steroidogenic enzymes, receptors, and binding proteins, thereby creating a low-estrogen, high-androgen environment that is diametric to the growth of endometriotic tissue.[2,3] However, the use of exogenous steroids or danazol is associated with adverse side effects.[4] Therefore interest has been spurred in the development of a therapy to manage endometriosis that does not elicit the undesirable side effects of previous therapies.

Most recently, gonadotropin-releasing hormone (GnRH) analogs have been developed for the management of endometriosis.[4,5] GnRH analogs cause a reversible block of ovarian cyclicity. In turn, this hypogonadal state causes the regression of ectopic endometriotic tissue by inhibiting endometrial proliferation. GnRH agonists exert their antigonadal activity by down regulation of the GnRH receptor but may elicit stimulation of the disease if administered during the follicular phase of the menstrual cycle. The GnRH antagonists are competitive inhibitors of GnRH receptor binding and therefore do not cause the initial stimulation that may be found with the GnRH agonist.

The development of the long-acting GnRH antagonist antide has generated the promise of a potential endometriosis therapy that is free of the adverse effects of earlier drug therapies.[6] We have shown that a rapid regression of endometriosis occurs in the rat after treatment with antide.[7] However, prolonged GnRH analog therapy has been associated with hypogonadal side effects. To compensate for these effects, concurrent administration of exogenous steroids has been suggested.[5,8,9] However, the addition of steroids could contradict the beneficial effects of the GnRH analog in reduction of the endometriosis.

Regardless of the type of therapy, endometriosis recurs in a large proportion of patients on completion of treatment. The recurrence of endometriosis after cessation of therapy has been poorly studied because of the difficulty in performing repeated surgical observations in women. The lack of randomized, controlled clinical trials has also clouded the evaluation of medical treatments for endometriosis.[10] Therefore experimental endometriosis has been developed in animal models to evaluate the effects of drugs on endometriosis.[7,11-13] We designed these experiments to monitor the regrowth of the ectopic endometriotic tissue after complete suppression by antide and to evaluate the effects of exogenous steroid administration on antide-suppressed endometriosis in the rat.

Material and methods

Animals. Mature female Sprague-Dawley rats (250 gm; Harlan, Madison, Wis.) exhibiting regular 4-day estrous cycles were housed in environmentally controlled room with a 14-hour light, 10-hour dark cycle. Ovarian activity was indirectly measured daily at 7:00 AM in all rats by examination of vaginal cytology.[14] Rats were allowed a 2-week acclimation period in the vivarium before any procedure was performed. The rats were maintained in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" and the protocol for use of these rats was approved by the University of Kentucky.

Endometriosis was surgically induced in the rats by autotransplantation of uterine tissues as previously described by Vernon and Wilson.[15] Briefly, 3 mm squares of uterine tissue (implants) were obtained from the ablation of the distal third of the right uterine horn. The implants were autotransplanted to the arterial cascades of the intestinal mesentery with 4.0 nylon suture. After 6 weeks (for recuperation and development of endometriotic implants) experimentation began.

Experiment 1: Spontaneous recurrence of endometriotic implants after antide suppression. Rats with surgically induced endometriosis ($n = 56$) were subcutaneously injected with the GnRH antagonist antide [*N*-Acetyl-D-2-Naphthyl^1,D-4-ChloroPhe^2,D-3-Pyridyl^3, Nicotinoyl - Lys^5,D - Nicotinoyl - Lys^6,Isopropyl - Lys^8,D-Ala^{10}] with a regimen previously shown to suppress ovarian and hypothalamic activity.[14] Antide was designed and synthesized by Drs. K. Folkers and C. Y. Bowers and made available for research by the contraceptive development branch of the National Institute of Child Health and Human Development. Rats received antide (2 mg/kg) in oil or oil alone (vehicle control) at 12:00 noon on day 0 (proestrus) and days 3, 6, and 9. Rats were killed at 12:00 noon on days 0, 6, 12, 18, 24, 30, and 42 ($n = 4$ antide-treated and 4 vehicle-treated rats killed per day). At the time of death, rats were anesthetized with ether and sera were collected by means of cardiac puncture. Serum estradiol and progesterone levels were determined in our laboratory. All serum steroid concentrations were measured within the same assay. The intraasay coefficients of variation for the estrogen and progesterone assays were 9.3% and 6.4%, respectively. The endometriotic implants were photographed in situ, measured, and weighed. The data presented here are reported as length because the measurements of length, width, and volume yielded the same experimental results.

Experiment 2: Steroid-modulated regrowth of antide-suppressed endometriosis. A second group of rats with surgically induced endometriosis received antide ($n = 20$) or vehicle ($n = 4$) as described in exper-

iment 1. On day 9, 1 hour after the last antide treatment, these rats received a subcutaneous methylcellulose pellet that contained either estradiol (0.5 mg), progesterone (25 mg), estrogen plus progesterone, cholesterol control (Innovative Research of America, Rockville, M.D.), or no steroid pellet. The reproductively cyclic vehicle-treated rats did not receive steroids.

The rats in this experiment were killed at 12:00 noon on day 12, and therefore had received 72 hours of in vivo steroid exposure. Rats were killed and sera were collected as described above. All serum steroid concentrations were measured within the same radioimmunoassay. The coefficients of variation within the estrogen and progesterone assays were 7.3% and 3.2%, respectively. Some of the steroid concentrations for experiment 2 only, were presented elsewhere in tabular form.[14] The results of these steroid assays have been reproduced in figure form to illustrate the impact of exogenous steroid supplementation on antide-suppressed endometriosis.

Statistical analysis. Statistical analysis of the endometriotic implant size and serum steroid concentrations was performed with Statistical Analysis Systems general linear models computer program (SAS Institute, Cary, N.C.). When the F test from the analysis of variance indicated significance ($p < 0.01$), Duncan's multiple range test was used to evaluate differences in the least squares means of the endometriotic implant size and serum hormone concentrations.

Results

Animals. The rat has a 4-day estrous cycle that is easily monitored by changes in the vaginal cells.[14] The vaginal cells of the vehicle-treated animals were indicative of the characteristic 4-day estrous cycle of the rat for the duration of the project. However, as previously reported,[7, 14] antide elicited a rapid and profound period of anestrus (predominantly polymorphonuclear cells with few epithelial cells) that began on day 2. In experiment 1, anestrus continued until day 30, 21 days after the last antide injection. In experiment 2, the antide-treated rats also displayed a profound acyclic period beginning at day 2. However, at day 12 the vaginal cells of the rats treated with antide and estradiol or antide and estradiol plus progesterone displayed a mixture of rounded and cornified epithelium that is similar to the vaginal cells of a proestrus to estrus stage of the estrous cycle. The vaginal cells of the rats treated with antide and progesterone, antide and cholesterol, and antide and no steroid remained anestrus in appearance at day 12.

Experiment 1: Spontaneous recurrence of endometriotic implants after antide suppression. Six weeks after the initial surgery the mean length (± SEM) of the endometriotic implants had increased from the original size at autotransplantation of 3.00 mm to 5.58 ± 0.42 mm (day 0; Fig. 1). In the absence of antide, implant size was maintained throughout the experiment. However, antide began to suppress endometriotic implant size by day 6 (3.17 ± 0.42 mm). Significant suppression was observed ($p < 0.01$) on days 12, 18, and 24 (0.58 ± 0.42 mm, 0.50 ± 0.30 mm, and 2.71 ± 0.30 mm, respectively). By day 30 (4.71 ± 0.30 mm) and day 42 (6.00 ± 0.30 mm), implant size had returned to pretreatment values (Fig. 1).

The reduction in length of the endometriotic implant was coincident to the antide-induced suppression of the ovarian steroids. In the vehicle-treated rats, serum estradiol and progesterone concentrations were characteristic of the stage of their estrous cycle at the time of death[15] (Figs. 2 and 3). Antide suppressed serum estradiol on days 6, 12, and 18 as compared with the vehicle-treated rats ($p < 0.01$; Fig. 2). Serum progesterone concentrations of the antide-treated rats were also suppressed by day 6 but, unlike the estradiol concentrations, remained significantly suppressed for the duration of the study ($p < 0.01$; Fig. 3).

Experiment 2: Steroid-modulated regrowth of antide-suppressed endometriosis. In experiment 2, the length of the endometriotic implants from the vehicle-treated rats at death (day 12) was 5.88 ± 0.35 mm (mean ± SEM, Fig. 4). The implants of the rats that received antide without steroid stimulation were significantly suppressed (0.48 ± 0.30 mm; $p < 0.01$; Fig. 4) as compared with the vehicle-treated and steroid-treated rats. The administration of the exogenous steroids (day 9) caused a treatment-dependent response in the implants of the antide-treated rats. An increase in implant size was found in the rats treated with antide and estradiol (2.29 ± 0.47 mm), antide and progesterone (1.48 ± 0.33 mm), and antide and cholesterol (1.83 ± 0.37 mm) as compared with the rats that received antide alone ($p < 0.01$; Fig. 4). However, the size of the implants of the rats treated with antide and estradiol, antide and progesterone, and antide and cholesterol was not as great as that observed in the implants of the vehicle-treated rats or rats treated with antide and estradiol plus progesterone (4.63 ± 0.94 mm). The implants of the rats treated with antide and estradiol plus progesterone had reverted to a size similar to that observed in the vehicle-treated group (Fig. 4).

The serum estradiol and progesterone concentrations of the vehicle-treated rats were typical of the steroid concentrations observed in rats at 12:00 noon on the afternoon of proestrus[16] (Fig. 5). Both serum estradiol and progesterone were suppressed ($p < 0.01$) in the rats treated with antide and no steroid as com-

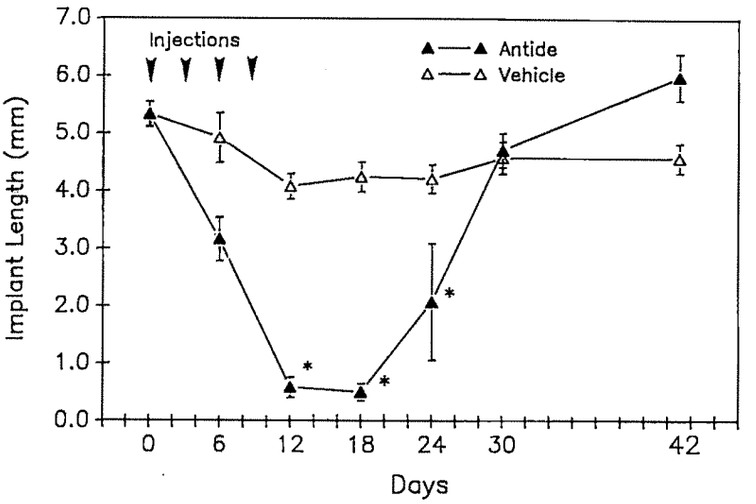


Fig. 1. Effect of antide on endometriotic implant length. Rats received four injections *(arrows)* of vehicle or antide over 9 days. Each point represents mean implant length ± SEM of six implants from each of four rats ($n = 24$ per point). Means marked with *asterisk* are statistically different from vehicle treatment ($p < 0.01$).

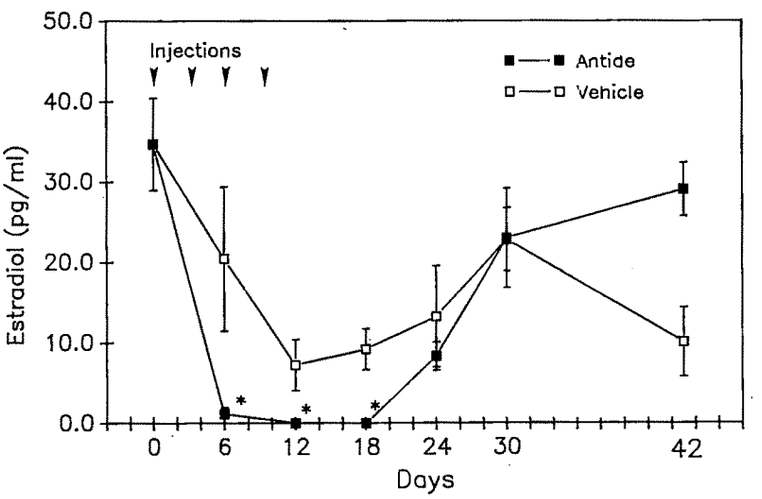


Fig. 2. Effect of antide on serum estradiol concentrations. Rats received four injections *(arrows)* of vehicle or antide over 9 days. Each point represents mean serum estradiol concentration ± SEM of four rats ($n = 4$ per point). Means marked with *asterisk* are statistically different from vehicle treatment ($p < 0.01$).

pared with all other treatments (Fig. 5). In the rats treated with antide and estradiol, serum estradiol concentrations were elevated ($p < 0.01$) as compared with those of rats that had not received estradiol treatment but not as great as those of the antide and estradiol plus progesterone. The serum progesterone concentrations of the rats treated with antide and estradiol were greater than those of the rats treated with antide and no steroid but similar to those of the vehicle-treated rats (Fig. 5). Serum estradiol remained suppressed ($p < 0.01$) in the rats treated with antide and progesterone. The serum progesterone concentrations of the rats treated with antide and progesterone were elevated ($p < 0.01$) as compared with those of the rats that did not receive progesterone treatment but similar to those of the rats treated with antide and estradiol plus progesterone (Fig. 5). Serum estradiol concentrations were greater ($p < 0.01$) in the rats treated with antide and estradiol plus progesterone than in all other groups. The serum progesterone concentrations of the rats treated with antide and estradiol plus progesterone were elevated ($p < 0.01$) as compared with those of the

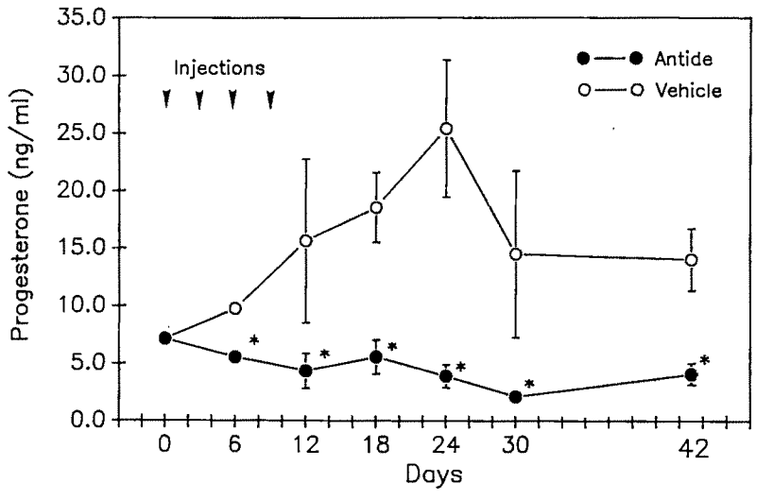


Fig. 3. Effect of antide on serum progesterone concentrations. Rats received four injections *(arrows)* of vehicle or antide over 9 days. Each point represents mean serum progesterone concentration ± SEM of four rats ($n = 4$ per point). Means marked with *asterisk* are statistically different from vehicle treatment ($p < 0.01$).

rats that had not received progesterone but similar to those of the rats treated with antide and progesterone (Fig. 5). In the rats treated with antide and cholesterol, serum estradiol was suppressed ($p < 0.01$) but serum progesterone was not different from the vehicle-treated rats (Fig. 5).

Comment

GnRH analogs have been used effectively in women for the treatment of endometrosis.[2, 4, 8] Although these analogs elicit regression of endometriotic tissue without the negative effects associated with earlier steroidal therapies, recurrence of the ectopic tissue occurs on cessation of the therapy. The mechanism of this recurrence is unclear. Previous research has not shown whether the growth of endometriosis observed after therapeutic suppression was renewed growth of the existing endometriosis or new disease. Part of the mystery that surrounds the mechanism of recurrent endometriosis in women stems from the ethical limitations in performing repeated laparoscopies. Therefore we have investigated the effects of the GnRH antagonist, antide, on endometriosis in the rat.

Antide, with its prolonged duration of action,[17] does have the potential to offer symptomatic relief by suppressing endometriosis as long as the drug exerts it antigonadal effects. We have previously shown that antide in the rat reduces the endometriotic implants to less than their original size at autotransplantation.[14] However, as we have shown here, this marked suppression of the endometriotic implants observed during antide therapy was spontaneously reversed within 3 weeks after the cessation of antide treatment or within 3 days after steroid replacement therapy. We did not observe a recurrence of the endometriotic implants while serum estrogen and progesterone concentrations were suppressed. Once the withdrawal from the suppressive effects of antide was complete and serum estradiol concentrations returned to physiologic levels, the regrowth of the endometriosis ensued. This resurgence of disease observed at the original sites of implantation in the rat suggests that recurrent endometriosis is a result of renewed growth rather than new disease. Therefore the potential transitory relief a patient may experience from the antide therapy would dissipate as regrowth of the endometriosis occurs. Others have shown a high clinical occurrence rate after GnRH agonist therapy was discontinued.[8] Hence, prolonged exposure to antide may be required to maintain remission of the endometriosis.

However, prolonged GnRH analog–induced hypogonadism has been associated with adverse side effects in women. These effects include hot flashes, headaches, vaginal dryness, dyspareunia, and a decrease in bone density that can ultimately lead to osteoporosis. To compensate for these side-effects, concurrent administration of exogenous steroids has been suggested. However, as the data presented here suggest, steroid add-back therapy could elicit regrowth of the GnRH analog–suppressed endometriosis.

Certain types of steroid replacement therapy abate the suppressive effects of GnRH analogs in the treatment of endometriosis.[18] We showed that steroid replacement markedly, but selectively, increased the endometriotic implant size in the antide-treated rats at a time (day 12) when the disease had not spontane-

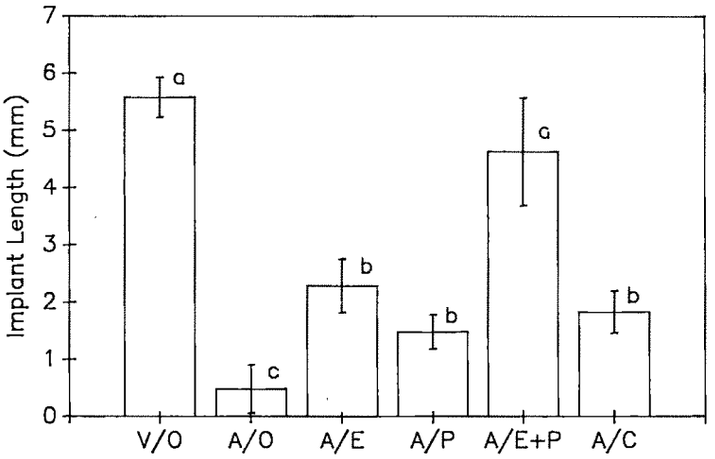


Fig. 4. Effect of steroid replacement therapy on endometriotic implant size of vehicle-treated and antide-suppressed rats. Vehicle treated rats *(V/O)* and antide only–treated rats *(A/O)* received no steroids. Remaining antide-treated rats received one of the four following steroid treatments: estrogen *(A/E)*, progesterone *(A/P)*, cholesterol *(A/C)*, or estrogen plus progesterone *(A/E + P)*. Means with different superscripts are significantly different from each other ($p < 0.01$; $n = 4$ per treatment group).

ously recurred in the rats that did not receive steroids. Estrogen plus progesterone replacement therapy elicited complete recurrence of endometriosis in antide-suppressed rats at 72 hours after the initiation of steroid replacement. A more subtle, yet significant, regrowth of the disease was observed when estradiol, progesterone, or cholesterol were administered individually. This differential response of the endometriotic implants to the steroid replacement therapies may have been a result of various mechanisms.

The substantial regrowth of the endometriotic implant observed when estradiol plus progesterone was given may be a reflection of the higher systemic concentrations of estradiol in this group. Although both the estradiol and estradiol-plus-progesterone groups received the same estradiol treatments, the serum estradiol concentrations were significantly greater in the latter group. These increased levels of serum estradiol might be attributable to peripherial aromatization of the exogenous progesterone to additional estrogenic compounds that elicited the enhanced regrowth. Although not studied here, further long-term exposure to estradiol could eventually cause a regrowth of the endometriosis similar to that observed with the estrogen-plus-progesterone treatment. In support of this idea, others have noted that long-term estrogen exposure will cause regrowth of endometriotic implant size in ovariectomized rats.[13] However, if peripheral aromatization of progesterone were occurring, one would expect to find increased estradiol concentrations in the progesterone-treated rats. No such increase in estradiol was found in the progesterone-treated rats.

Alternatively, one might speculate that the demonstrative rebound of the endometriotic implants in the estrogen plus progesterone–treated rats may be attributed to the presence of both estradiol and progesterone, which more closely mimicked a natural, in situ steroidal environment than either of the steroids alone. This idea is also supported by the fact that the estradiol-plus-progesterone regimen also induced changes in the vaginal cytology that were more indicative of a cyclic animal. However, the exact reason for the increased serum estradiol concentrations and hence the increased rate of growth of the endometriosis in the estradiol plus progesterone–treated rats cannot be discerned from these studies.

In the antide-treated animals that received progesterone (without estrogen), an increase in implant growth was also observed. The exogenous progesterone may have had estrogenic effects in vivo. Others have reported increased uterine weights in rats given progestins, indicating an estrogenic action.[19] However, no estradiol was found in the serum samples of the progesterone-treated rats, suggesting an alternate mechanism for stimulation of implant growth. The progesterone treatment might have increased the secretory capacity and elicited decidualization of the ectopic endometrium. These changes could be responsible for the accumulation of intracellular and extracellular fluids within the cystlike endometriotic implants thereby causing an increase in their size. On the other hand, prolonged subphysiologic levels of progesterone do not hinder the recurrence of the endometriosis after cessation of antide therapy. Spontaneous recurrence of the antide-suppressed endometriosis was observed (experiment 1) even though progesterone concentra-

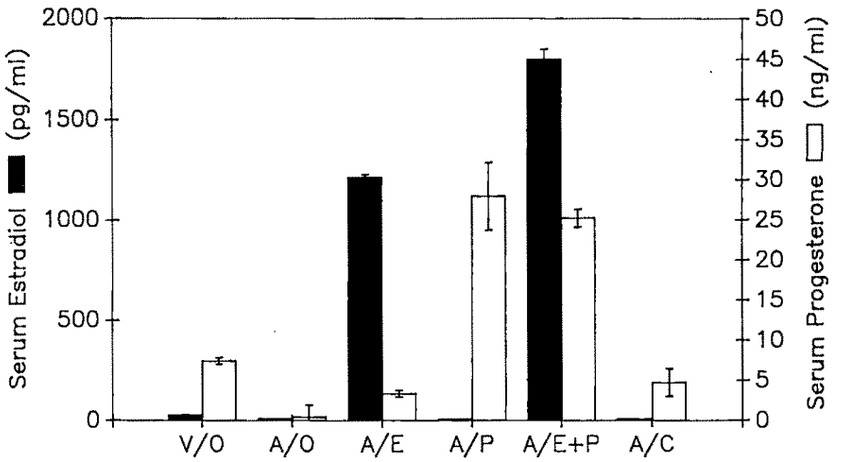


Fig. 5. Serum estrogen *(left axis)* and progesterone *(right axis)* concentrations after steroid replacement in antide-treated rats. Vehicle-treated rats *(V/O)* and antide only–treated rats *(A/O)* received no steroids. Remaining antide-treated rats received one of the four following steroid treatments: estrogen *(A/E)*, progesterone *(A/P)*, cholesterol *(A/C)*, or estrogen plus progesterone *(A/E + P)* ($n = 4$ per treatment group). Significant elevation ($p < 0.01$) in serum steroid concentration was observed in animals that received exogenous estradiol or progesterone *(A/E, A/P,* and *A/E + P)* relative to controls *(V/O* and *A/O)*.

tions remained diminished. These observations fit well with previous work that showed that the progesterone-induced stimulation of endometriosis with or without GnRH analog–therapy is dependent on both the type of progestin used and the regimen by which it is administered.[8-9, 18]

The regrowth of the endometriotic tissue observed in the antide-treated rats after the administration of cholesterol was unexpected. The serum progesterone of the cholesterol-treated rats was similar to the vehicle-treated rats, indicating that the cholesterol could have been metabolized to progesterone in the adrenal glands or ovaries. The administration of antide may have altered the cholesterol or steroid metabolism of these rats. Another explanation for the increased serum progesterone of this group was that the cholesterol pellet was contaminated with progesterone. However, analysis of these pellets in our laboratory showed that this was not the case. Although unlikely, the possibility exists that the methylcellulose itself may have been responsible for the effects seen in the cholesterol group. The mechanism of endometriotic implant regrowth after cholesterol treatment requires further investigation.

The benefits of GnRH analog–treatment for endometriosis-associated infertility remain to be confirmed. Use of the GnRH agonist, nafarelin, has indicated a potential for increased fecundity rates similar to those seen in endometriosis patients treated by conservative surgery or danazol therapy.[20] However, at doses sufficient to suppress endometriosis, we previously showed that the antagonistic analog antide generates follicular atresia and infertility in the rat.[14] It is obvious that care must be taken when results are extrapolated across species, but these observations of spontaneous recurrence of endometriosis along with the previously reported antide-induced infertility justify further investigation into the mechanisms of action of this GnRH antagonist on both endometriosis and folliculogenesis.

In summary, although antide elicits a marked suppression of endometriosis in the rat, regrowth of the disease ensues with cessation of therapy. Furthermore, steroid replacement therapy stimulates the rapid recurrence of the endometriotic implant growth. If also present in women, this persistence of endometriosis may partially explain treatment failure and recurrence of the disease after hormone therapies.

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Diastolic circulatory dynamics in the presence of elevated placental resistance and retrograde diastolic flow in the umbilical artery: A Doppler echographic study in lambs

Jean-Claude Fouron, MD, Georges Teyssier, MD, Enrique Maroto, MD, Manon Lessard, RT, and Gerald Marquette, MD

Montreal, Quebec, Canada

To evaluate the effects of elevated placental resistance on the diastolic blood flow in the main arteries of the fetus, placental resistance was mechanically increased in six exteriorized lambs. A string was inserted into the exposed section of the umbilical cord and placed around the veins isolating them from the arteries. Doppler flow velocities waveforms were measured by placing a lightweight acoustic bag on the umbilical cord. Placental resistance was increased by tightening the string until retrograde diastolic flow was observed in the umbilical artery. All measurements were obtained within 10 minutes. Heart rate, Po_2, Pco_2, and pH before umbilical vein compression were 177 ± 24 beats/min, 16.4 ± 1.8 mm Hg, 42.5 ± 4 mm Hg, and 7.3 ± 0.05, respectively, and 112 ± 38 beats/min, 5.9 ± 1.5 mm Hg, 56.6 ± 7.0 mm Hg, and 7.2 ± 0.05, respectively, after umbilical vein compression. The patterns of diastolic flow observed after compression were descending aorta, retrograde; aortic arch, retrograde; ascending aorta, bidirectional; cephalic aorta, forward, which were quite different from their respective baseline patterns. It is concluded that the appearance of reverse diastolic flow in the umbilical artery indicates that (1) the lowest vascular resistance in the fetal circulatory network is no longer at the placental but at the cerebral level, and (2) preplacental blood with low oxygen content from the descending aorta and pulmonary artery is being shifted toward the brain. (Am J Obstet Gynecol 1991;164:195-203.)

Key words: Fetus, Doppler ultrasonography, umbilical circulation

There is convincing evidence that shows that an increase in placental resistance is associated with a decrease and sometimes a complete disappearance of the diastolic forward flow normally observed in the umbilical artery.[1, 2] In severe cases, a reversal of this diastolic flow has even been observed.[3] Whereas the absence of diastolic flow can be explained by an increase in placental vascular impedance, no satisfactory explanation has been given for the retrograde flow documented in very ill fetuses. A rebound phenomenon against a high resistance can hardly explain this low velocity flow that frequently occupies the entire diastole and is recorded not only in the umbilical circulation but also in the descending aorta.[4, 5] Whereas in systole the progression of blood flow is primarily ensured by the driving force of the cardiac pump, in diastole the main factor left to influence the distribution of the blood into the various vessels is the peripheral resistance. Therefore this Doppler echographic study was planned with the hypothesis that a more defined assessment of the orientation of the blood stream in the main fetal arteries during diastole could provide some insight on the changes in the peripheral resistances and circulatory dynamics resulting in umbilical diastolic retrograde flow.

From the Fetal Cardiology Unit, Pediatric Cardiology Division, Department of Pediatrics, and the Department of Obstetrics and Gynecology, Sainte-Justine Hospital, University of Montreal.
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Reprint requests: Jean-Claude Fouron, MD, Division of Cardiology, Sainte-Justine Hospital, 3175 Côte Sainte-Catherine, Montreal, Quebec, Canada H3T 1C5.
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Material and methods

This project was approved by the Ethical Committee for the care and use of animals of our institution. Six fetal lambs were used in this experiment. The gestational age of each lamb was >140 days. The ewes were anesthetized with alternating intravenous injections of ketamine hydrochloride (3 mg/kg) and sodium pentobarbital (Nembutal) (2 mg/kg) every 20 to 40 minutes. The uterine wall was first marsupialized to the border of the surgical abdominal wound. This precautionary measure ensured that the uterus remained within the abdominal cavity throughout the experiment. Great care was taken to prevent torsion or undue traction of the uterine horn, thereby ensuring an adequate blood flow through the uterine artery. The hind limb of the fetus was first exteriorized allowing can-

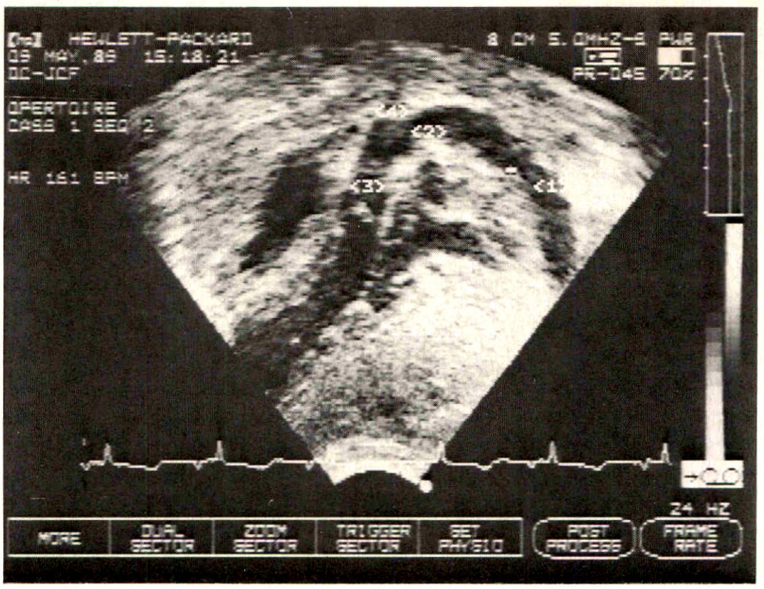


Fig. 1. Real-time echographic imaging of left ventricular outflow and of aorta recorded from left subxiphoid approach. Numbers correspond to sites where Doppler assessment of diastolic blood flow patterns were carried out. **1,** Descending aorta; **2,** aortic arch; **3,** ascending aorta; and **4,** cephalic aorta.

nulation of one of the femoral arteries for blood gas analysis and arterial pressure recordings in the descending aorta. This was followed by complete exteriorization of the fetus, which was required for the Doppler echographic investigation. The head of the fetus was put into a rubber bag containing warm saline solution to prevent breathing. The animal was kept very close to the laparotomy incision, keeping most of the umbilical cord inside the uterine cavity to minimize tension. Only a few centimeters of the umbilical cord were used for the flow velocity measurements. This section was regularly humidified with warm saline solution. Fetal temperature was kept constant between 38.5 and 39.5° C with the help of a heating lamp.

Ultrasonographic evaluations were obtained with a Sonos-100 HP echocardiograph equipped with real-time and pulsed Doppler echocardiographic capabilities. Doppler investigation was guided by two-dimensional imaging of the vascular structures with a 5 MHz mechanical transducer. The Doppler flow velocity studies were based on determinations made through the left subxiphoid approach. From this approach the ascending aorta, the bifurcation between the cephalic branch and the aortic arch, and the descending aorta could be well visualized (Fig. 1). Doppler flow velocity waveforms in the umbilical artery were recorded with the help of a standoff acoustic bag. Great care was taken to avoid any compression of the cord, which was surrounded by its natural Wharton jelly. The acoustic bag was made in our laboratory by filling one finger of a fine rubber glove with warm acoustic jelly and gently placing it over the umbilical cord. The absence of compression or vessel reaction was reflected by the presence of a significant diastolic flow on the Doppler flow velocity waveform.

Arterial pressure was constantly recorded during this procedure with a Cambridge recorder. Arterial pH, Po_2, and Pco_2 were analyzed on samples of 0.8 ml of blood with a blood gas analyzer from Instrument Laboratory Equipments.

Experimental protocol. The animals were considered to be in a basal state when two consecutive blood samples and pressure recordings taken 5 minutes apart showed blood gas values, arterial pressure, and heart rate as being stable and within normal physiological limits. Flow velocity waveforms in the umbilical artery were then recorded (Fig. 2, *A*), followed by recordings in the ascending aorta, cephalic aorta, aortic arch, and descending aorta. The recordings were obtained as quickly as possible to achieve a complete assessment of the diastolic patterns within 10 minutes. To ensure that the measurements were recorded during the same acute condition (similar degree of placental resistance), umbilical artery blood flow velocity waveforms were recorded immediately after each series of Doppler interrogations and compared with the results obtained before the commencement of the scannings.

To create high placental resistance, a small umbilical

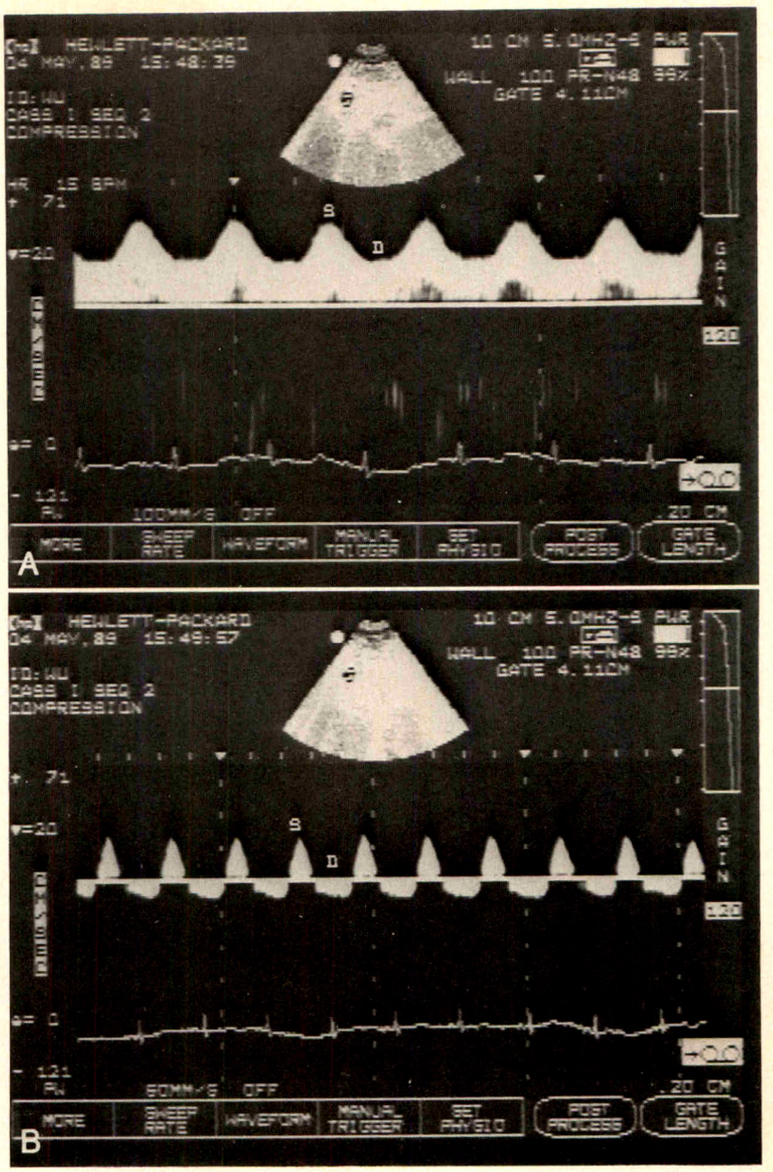


Fig. 2. Umbilical artery velocimetry. **A,** Before umbilical vein compression, showing forward flow both in systole *(s)* and diastole *(d)*. **B,** During umbilical vein compression, showing reverse flow in diastole *(d)*.

string was gently inserted through the Wharton jelly directly between the arteries and the veins thereby separating them from each other. While the baseline data were collected the string was loosely tied, not exerting any pressure, around the veins and fixed with a pair of small hemostatic forceps. After the basal measurements were recorded, the umbilical veins were mechanically compressed by tightening the string. Doppler flow velocity monitoring was continuous throughout the basal and compression conditions. Because the aim of the study was to assess the peripheral vascular resistance during reverse umbilical flow, the procedure was stopped immediately when retrograde diastolic flow was detected in the umbilical artery (Fig. 2, *B*). Flow velocity measurements were then carried out on the same vascular sites described in the basal state. Blood samples were withdrawn for blood gas analysis toward the end of the Doppler recordings.

Data analysis. The diastolic flow patterns in each of the vascular sites evaluated were classified according to the direction of the blood stream. Thus the diastolic flow could be described as forward when in the same

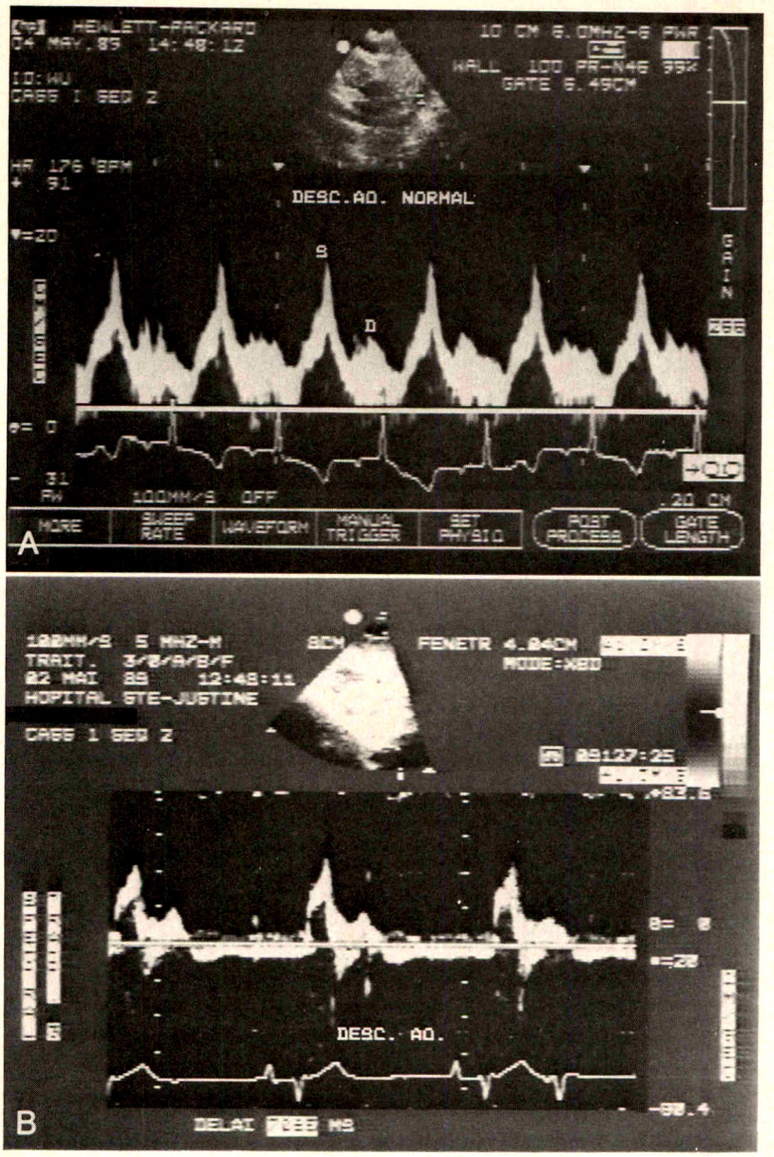


Fig. 3. Velocimetry in the descending aorta. **A,** At basal condition, forward flow is recorded in diastole. **B,** During umbilical vein compression, diastolic reverse flow is observed.

direction as systolic flow during the entire (forward total) or part (forward partial) of diastole; retrograde when in the opposite direction compared with systolic flow during the entire (retrograde total) or part (retrograde partial) of diastole; absent when no Doppler signals could be recorded in diastole; and bidirectional when Doppler signals could be recorded alternating between forward and retrograde during the same diastolic period.

Pulsatility index was calculated in the umbilical artery as described by Gosling.[6] Blood gas values, arterial pressures, and pulsatility indexes obtained during normal and increased placental resistance were compared with the paired Student *t* test.

Results

Basal condition (low placental resistance). The blood gas values, arterial pressures, heart rate, and pulsatility indexes are given in Table I. All six animals had the same patterns of diastolic blood flow. They were as follows: umbilical artery, forward total (Fig. 2, *A*); descending aorta; forward total (Fig. 3, *A*); aortic arch,

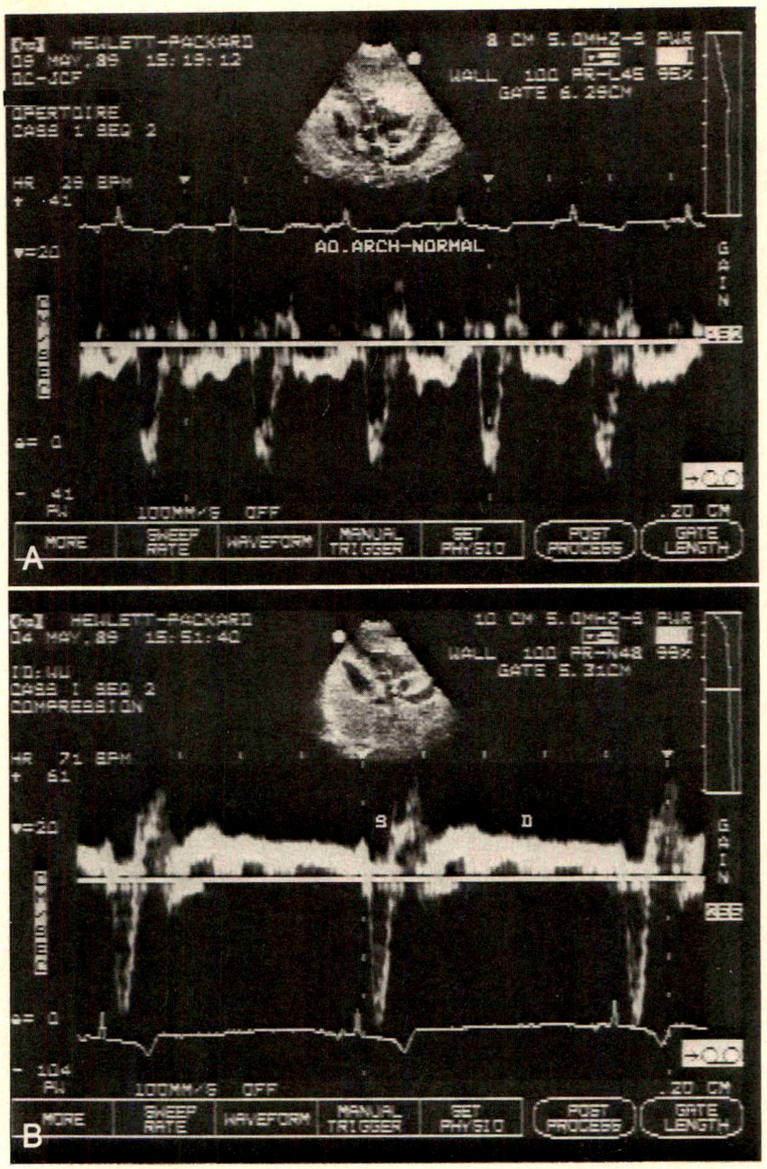


Fig. 4. Velocimetry in aortic arc. **A,** At basal condition, forward diastolic flow. **B,** Retrograde flow during umbilical vein compression.

forward total (Fig. 4, *A*); ascending aorta, bidirectional (Fig. 5, *A*); cephalic aorta, bidirectional (Fig. 6, *A*).

Umbilical vein compression (high placental resistance). Temperature, arterial pressure, and blood gas values were monitored at very close intervals. Table I shows the blood gas values and arterial pressures in the descending aorta, along with heart rate and umbilical artery pulsatility indexes obtained during umbilical vein compression. A marked drop of P_{O_2} and a moderate rise of P_{CO_2} were observed when compared with the basal state ($p < 0.01$). The pH did not change significantly. Whereas arterial pressures increased above baseline values immediately after umbilical vein compression, no statistical significance was reached. As noted in Table I, the pulsatility index reached an average of 1.82 in the umbilical artery after compression, which was statistically significant from the basal state value of 0.58. A marked bradycardia was observed during compression, and this difference between the normal (basal state) and the abnormal (during mechanical compression of the umbilical veins) heart rate values was found to be statistically significant. The patterns of diastolic flows were the same for all six animals: umbilical artery, retrograde total (Fig. 2, *B*); descending

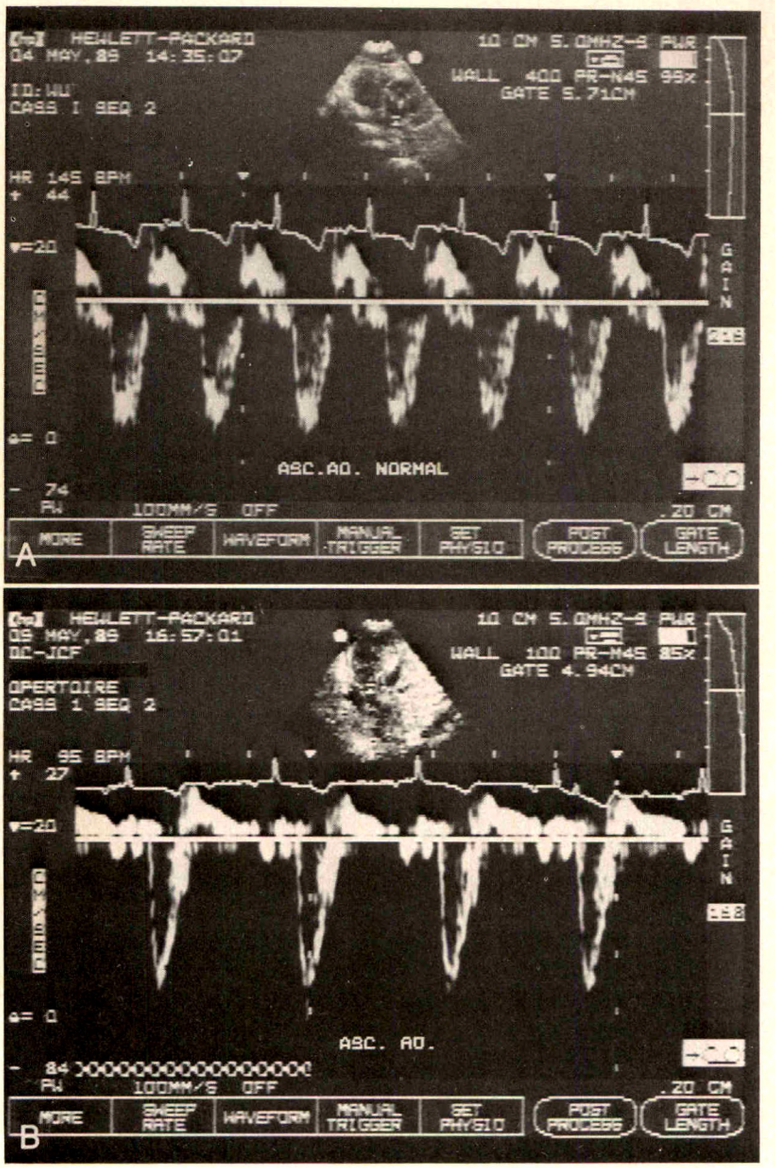


Fig. 5. Velocimetry in ascending aorta shows bidirectional diastolic flow both before **(A)** and during umbilical vein compression **(B).**

aorta, retrograde total (Fig. 3, *B*); aortic arch, retrograde total (Fig. 4, *B*); ascending aorta, bidirectional (Fig. 5, *B*); cephalic aorta, forward total (Fig. 6, *B*). Thus the cephalic aorta was the only main artery where forward flow was recorded during the entire diastole.

Comment

The application of the Doppler echographic technique to the assessment of blood flow velocities in the fetus is hampered by factors such as difficulties in obtaining the proper angle of insonation, a moving target, the size of the vessels that require a very small sample volume, and the distance between the transducer and the blood vessels. The protocol of our investigation added another element to these limiting factors by requiring a Doppler flow velocity sampling in five different sites along the systemic circulation during a relatively short period of time (<10 minutes). Preliminary trials confirmed that satisfactory data could not be collected on a chronic preparation with the transabdominal ultrasonograph approach because it was never possible to obtain a proper recording in all five vascular sites within the relatively short time period required by the protocol. Therefore we elected to obtain the desired information on an exteriorized fetus. Stress and fetal deterioration associated with this approach have been

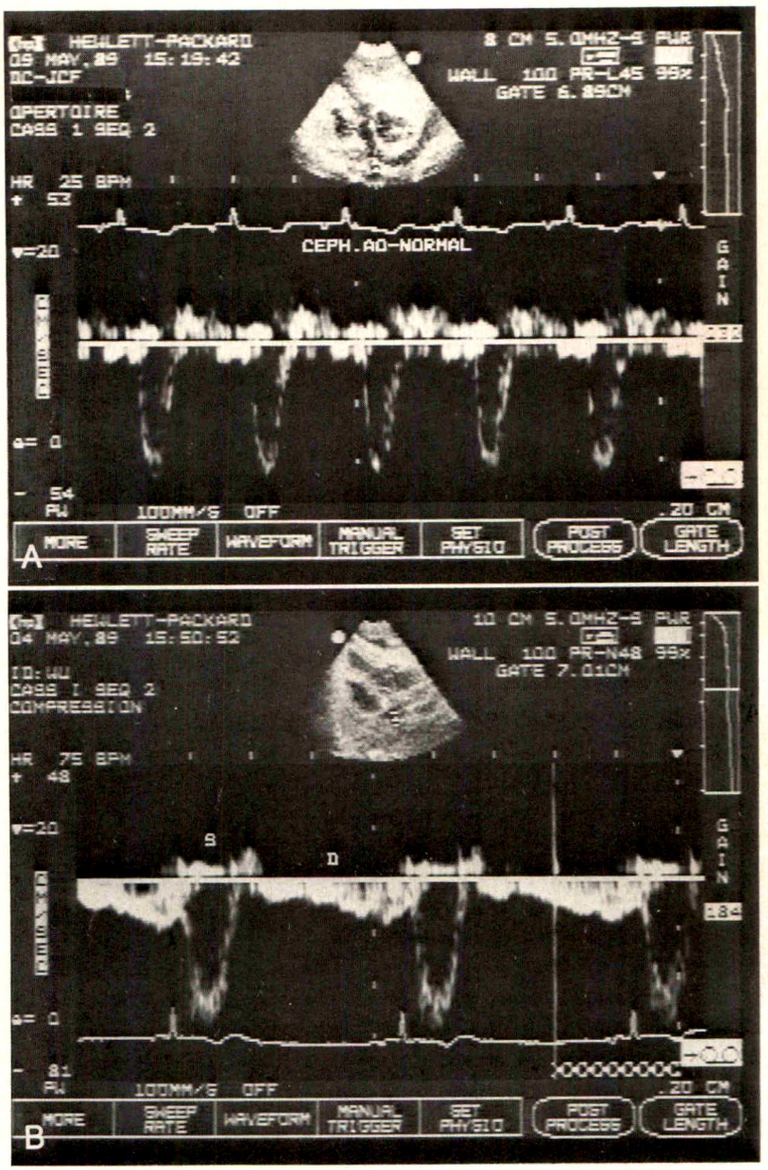


Fig. 6. Blood flow velocity waveforms in cephalic aorta. **A,** In diastole forward and retrograde flows are normally observed. **B,** With elevated placental resistance, only forward flow is recorded during entire diastole.

well described.[7] To minimize these drawbacks the duration of the entire experiment was kept to a minimum, and great care was taken to keep the uterus and the umbilical cord in the maternal abdominal cavity throughout the experiment. Previous attempts by other investigators with the same approach have given satisfactory results.[8]

Diastolic events in a given circulatory bed are greatly influenced by the status of the peripheral vascular resistance. In a normal fetus the peripheral circulation is characterized by two specific elements: a high pulmonary and a low placental vascular resistance. In fact, the vascular network with the lowest resistance in the entire circulatory system is at the level of the placental vascular bed. Doppler flow velocity recordings have confirmed this fundamental aspect of fetal circulation by documenting the presence of a significant forward diastolic flow in the umbilical artery. The low placental resistance affects the direction of blood flow during diastole in the descending aorta and as far as the aortic arch. Consequently, any change in placental vascular resistance is bound to profoundly disturb the normal dynamics of the fetal circulation. Therefore a progressive increase in placental resistance should cause a

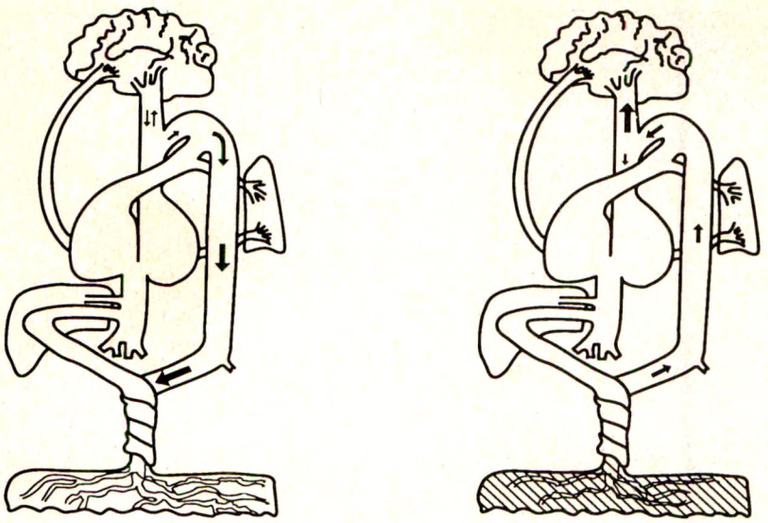


Fig. 7. Schematic representation of fetal diastolic flow distribution in presence of normal *(left)* and increased *(right)* placental vascular resistance.

Table I. Blood gas values and pressures in the descending aorta, and pulsatility indexes of the umbilical artery during normal and mechanically produced abnormal placental resistance

Placental resistance	*Blood gas values (mm Hg)*			*Pressure (mm Hg)*			*Pulsatility index*	*Heart rate*
	Po_2	Pco_2	*pH*	*Systolic*	*Diastolic*	*Mean*		
Normal	16.4 ± 1.8	42.5 ± 4.0	7.3 ± 0.05	59 ± 6	43 ± 6	48 ± 7	0.58 ± 0.23	177 ± 24
Abnormal	5.9* ± 1.5	56.5* ± 7.0	7.2 ± 0.05	67 ± 15	47 ± 14	53 ± 14	1.82* ± 0.40	112* ± 38

*Significant difference from normal values ($p < 0.05$).

corresponding decrease of the forward flow in the aorta and the aortic arch where complete disappearance of any significant blood flow in diastole can be observed. However, any further increase in placental resistance will then create an altered hemodynamic state in which the lowest vascular resistance is no longer at the placental level, but rather at the cerebral level. Indeed, in the presence of hypoxemia, as a result of the decrease in placental blood flow, vasodilation of the cerebral vessels will occur. The rise in Pco_2, a consequence of placental insufficiency, also promotes further cerebral vasodilation. When diastolic retrograde flow is observed in the umbilical artery, this indicates that during diastole the blood is actually flowing from the umbilical artery, the descending aorta, and the aortic arch toward a lower resistance—the vascular channels of the cerebral circulation. A schematic representation of this altered hemodynamic state is illustrated in Fig. 7. Analogous situations are known to occur in extrauterine life in cases with cerebral arteriovenous fistula.[9] Reverse diastolic flow from the descending aorta to the pulmonary artery has also been well documented in infants with patent ductus arteriosus and left-to-right shunting.[10]

Bradycardia was observed in all fetuses during umbilical vein compression. This was most likely related to baroreceptor activation by the arterial pressure rise in response to the change in systemic vascular resistance. Whereas bradycardia is known to increase the pulsatility index, it usually does not do so to the point of complete disappearance of diastolic forward flow, and it most certainly does not produce retrograde flow. This is exemplified in fetuses with complete arteriovenous block and marked bradycardia in which diastolic reverse flow in the umbilical artery was never observed in our experience. We do not think that bradycardia alone can explain the results observed in this study. Therefore for retrograde flow to occur in the umbilical artery another vascular bed somewhere in the circulatory system must have a lower impedance than the placental vascular system.

In the fetus a reverse diastolic flow from the descending aorta and umbilical artery toward the cerebral circulation would have disastrous effects on tissular oxygenation. Blood going in retrograde fashion through the aortic arch is then made of preplacental red cells, low in oxygen content, deviated from their normal route, the placental circulation. In these circumstances

the so-called "brain saving reflex" characterized by a cerebral vasodilation in response to hypoxemia[11, 12] could actually be detrimental to the brain. Indeed, vasodilation of the cerebral vessels should hasten the inversion of the relationship between placental and cerebral resistances. In our anesthetized animals this factor was probably not much of a determinant because pentobarbital has a depressing effect on the contractile response of the cerebral arteries.[13] However, this does not change the actual mechanism by which diastolic retrograde flow occurs. It can be speculated that without induction of anesthesia, as is most often the case in clinics, hypoxic active cerebral vasodilation will occur and reverse diastolic flow will be observed at a lower level of placental vascular resistance than the maximal resistance created during this study.

These data were collected from acute experiments on anesthetized animals. Further investigations should be carried out to evaluate their clinical implications. If confirmed in human beings, the hemodynamic events reported in this experimental study would certainly explain why the appearance of reverse diastolic flow in the umbilical artery and the descending aorta is considered a sign of impending fetal death.[14]

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Forskolin and methotrexate induce an intermediate trophoblast phenotype in cultured human choriocarcinoma cells

Robert N. Taylor, MD, PhD,[a] Erin D. Newman,[a] and Shiuan Chen, PhD[b]

San Francisco and Duarte, California

The human placenta and its associated membranes are vital to the maintenance, nutrition, and protection of the developing fetus. During placental development some cytotrophoblasts give rise to the chorionic membrane whereas others fuse to form a differentiated syncytium of cells that are responsible for placental protein and steroid hormone production. The mechanisms involved in the differentiation of the trophoblasts are unknown; however, an intermediate stage with a characteristic phenotype has been documented in vivo. We have observed that chemically dissimilar xenobiotic agents induced BeWo choriocarcinoma cells to change their usual cytotrophoblastic phenotype and acquire morphologic and functional characteristics typical of intermediate trophoblast. Incubation of BeWo cell cultures in the presence of 1 μmol/L methotrexate for 48 hours stimulated human chorionic gonadotropin secretion (2.3-fold) and aromatase activity (4.9-fold). Morphologic findings associated with these hormonal changes, including increased nuclear size and cytoplasmic expansion, were also observed. With the use of a computer-interfaced image analyzer, planimetric morphometry of the nuclear area of the cells revealed a 1.8-fold increase after incubation with methotrexate. The effect of forskolin, a direct activator of adenylyl cyclase, was also evaluated by means of this model of cytotrophoblast differentiation. The addition of 10 μmol/L forskolin to BeWo cultures also resulted in dramatic changes in trophoblast cell phenotype. Increases in human chorionic gonadotropin synthesis (7.3-fold), aromatase activity (13.5-fold), and nuclear area (3.0-fold) were induced over those of untreated cells. In addition, increases in [^{3}H]thymidine incorporation (1.7-fold) were afforded by both treatments. These results suggest that biochemical and cytologic changes associated with human trophoblast differentiation can be induced in vitro via activation of the adenylyl cyclase pathway by forskolin and through unknown and apparently independent signals by methotrexate. (Am J Obstet Gynecol 1991;164:204-10.)

Key words: Intermediate trophoblast differentiation, cyclic adenosine monophosphate, methotrexate

The early development of the human placenta is initiated by the differentiation of trophectoderm cells at the periphery of the preimplantation blastocyst. During implantation, undifferentiated mononuclear cytotrophoblast cells derived from the trophectoderm proliferate to form an invasive shell of tissue, which anchors the pregnancy to the uterine stroma. During the process of placental development, cytotrophoblasts differentiate and fuse, forming a syncytium of trophoblast that covers the placental villi.[1, 2] The process of trophoblast differentiation appears to involve a morphologic intermediate or transitional cell, which is the immediate precursor of syncytial trophoblast and has distinctive biochemical and cytologic characteristics.[2, 3] Syncytiotrophoblast is the primary site of synthesis and secretion of the major protein and steroid hormones of the placenta.[4] The reprogramming of cytotrophoblast cells with proliferative and invasive activities to establish a terminally differentiated syncytium with endocrine function is accomplished via unknown mediators. It is known, however, that cyclic adenosine monophosphate (cAMP)–mediated processes are involved in the promotion of human chorionic gonadotropin (hCG) and estrogen secretion in normal and malignant trophoblast cells.[5-8]

In this report we have examined a model of trophoblast differentiation using a human cell line (BeWo) derived from a choriocarcinoma.[9] Friedman and Skehan[10] were the first to show that BeWo cells with cytotrophoblast-like features under basal conditions could be induced to acquire syncytiotrophoblastic ultrastructure and behavior when cultured in the presence of 1 μmol/L methotrexate. The pathways by

From the Department of Obstetrics, Gynecology and Reproductive Sciences, Reproductive Endocrinology Center, University of California, San Francisco,[a] and the Division of Immunology, City of Hope National Medical Center, Duarte.[b]

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Reprint requests: Robert N. Taylor, MD, PhD, W.M. Keck Laboratory of Reproductive and Developmental Molecular Biology, Department of Obstetrics, Gynecology and Reproductive Sciences, M-1489, University of California, San Francisco, San Francisco, CA 94143-0132.

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which this antimetabolite effects such a transformation are unknown. Using this model we have demonstrated that two dissimilar xenobiotic agents, methotrexate and forskolin, act at a pretranslation level of regulation to induce an intermediate trophoblast phenotype in vitro.

Material and methods

Cell culture. BeWo choriocarcinoma cells (A63643) were obtained from the University of California, San Francisco, Cell Culture Facility and were maintained in RPMI 1640 media supplemented with 10% fetal calf serum as described by Friedman and Skehan.[10] Cells were plated at a density of 15,000 cells per well in 24-well Falcon dishes (Becton Dickinson, Lincoln Park, N.J.) and cultured for 2 days until each well was ~50% confluent. The media were aspirated and replaced with serum-free media containing forskolin, methotrexate, or no additive for 48 hours. Serum-free medium was prepared with RPMI 1640 supplemented with 20 mmol/L HEPES buffer (N-[2-hydroxyethyl]piperazine-N'[2-ethanesulfonic acid]), insulin (1 μg/ml), transferrin (5 μg/ml), bovine serum albumin (500 μg/ml), and penicillin-streptomycin (100 U/ml) obtained from Sigma (St. Louis). Unless indicated otherwise, the concentrations of forskolin and methotrexate were 10 and 1 μmol/L, respectively, which consistently gave near maximal responses of the morphologic and biochemical features described. Dibutyryl cAMP was used at a concentration of 1 mmol/L. At the end of each experiment, cells were trypsinized from representative wells and their numbers determined with a hemacytometer.

Cytologic analysis. Cells grown on plastic coverslips were fixed with 4% paraformaldehyde in phosphate-buffered saline solution (pH 7.4), stained with toluidine blue, and examined by light microscopy. Photomicroscopy was performed on a Leitz Ortholux II microscope equipped with a Vario Orthomat camera (E. Leitz, Rockleigh, N.J.). Planimetric morphometry was used to determine nuclear area with a Zeiss interactive digital analysis system (Zidas; Carl Zeiss, Thornwood, N.Y.). The nuclear areas of 150 cells were quantified.

[^{3}H]Thymidine incorporation. Cells grown for 48 hours in serum-free media in the absence (control) or presence of forskolin or methotrexate were pulsed for the last 4 hours of culture with 1 μCi/ml [^{3}H]thymidine (New England Nuclear, Boston). The incorporation of labeled nucleotide into 10% trichloroacetic acid–precipitable counts was determined by liquid-scintillation counting as described.[11]

hCG secretion. The secretion of hCG into the media of each culture was quantified with a specific double-antibody radioimmunoassay for the β-subunit described previously.[12] The sensitivity of this assay was 2 mIU/ml.

Northern analysis. BeWo cell ribonucleic acid (RNA) was extracted with guanidine thiocyanate (Fluka Chemie, Buchs, Switzerland) and purified over cesium chloride gradients.[13] hCG-β and aromatase messenger RNA (mRNA) accumulation in cultured BeWo cells was determined by electrophoresis of 10 μg aliquots of total cellular RNA after denaturation with glyoxal and dimethylsulfoxide as described by Thomas.[14] After electrophoresis, the 1% agarose gels were assessed by ultraviolet shadowing against a silica gel plate to ascertain that each lane contained equal amounts of ribosomal RNA. The RNA was transferred to nitrocellulose and hybridized with complementary DNA (cDNA) probes labeled with [α-^{32}P]2′-deoxycytidine 5′-triphosphate (~3000 Ci/mmol; New England Nuclear) and a random-primer kit (Boehringer-Mannheim, Indianapolis). The hCG-β cDNA (0.6kb)[15] was generously provided by Drs. Karen Talmadge and John Fiddes (California Biotechnology, Inc., Mountainview, Calif.). The human aromatase cDNA (2.4 kb) was described previously.[16]

Aromatase activity. The capacity of cells to synthesize estrogens was assessed by quantifying the enzymatic conversion of saturating concentrations of [1β, 2β-^{3}H]androstenedione to tritiated water and estrogen.[17] Briefly, BeWo cells exposed to control media or media containing forskolin (10 μmol/L) or methotrexate (1 μmol/L) for 48 hours were rinsed twice with phosphate-buffered saline solution and then incubated in 1 ml RPMI 1640 medium containing 1 μmol/L [1β,2β-^{3}H]androstenedione (diluted to a final specific activity of 5 Ci/mmol; New England Nuclear) for one hour at 37° C. The plates were transferred to an ice bath, the media were removed and extracted with chloroform, and the aqueous phases were chromatographed over minicolumns of charcoal and Celite and analyzed for tritium by scintillation counting. The cells were trypsinized from their respective wells and counted with a hemacytometer to normalize aromatase activities.

cAMP content. After 48 hours of incubation in control, forskolin-containing, or methotrexate-containing media, BeWo cells were solubilized and extracted with 0.1N hydrochloric acid. Cellular cAMP was acetylated by incubation in the presence of triethylamine/acetic anhydride, and its content was determined with a specific radioimmunoassay for cAMP.[18] Rabbit antiserum to cAMP was obtained from Chemicon (El Segundo, Calif.).

Statistical analyses. Data are presented as the mean ± SEM of replicate experiments. The treatment and control groups were compared by use of two-tailed analysis of variance. Where analysis of variance suggested that the treated cells had elevated phenotypic markers, specific treatments were compared against the

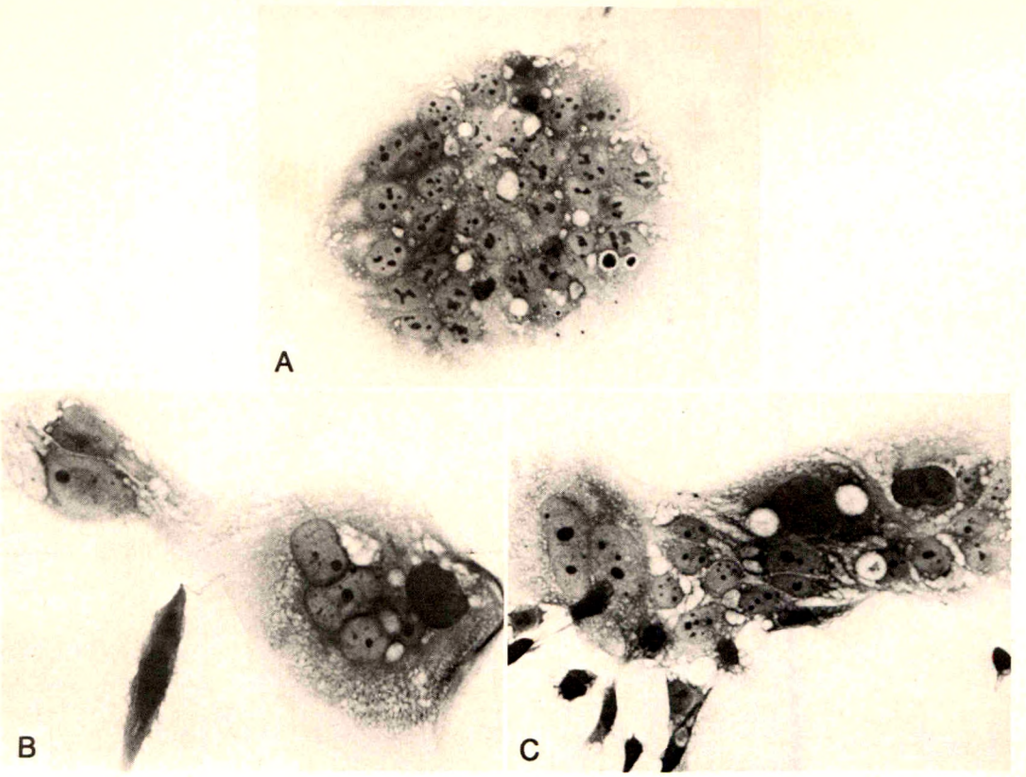


Fig. 1. Cytologic changes induced in BeWo cells. **A,** Control cells cultured in serum-free media for 48 hours contained uniform, small nuclei. **B,** Forskolin (10 μmol/L) exposure for 48 hours was associated with cytoplasmic and nuclear enlargement with prominence of nucleoli. **C,** Phenotypic changes similar to those seen in **B** were also noted after 48 hours of incubation with 1 μmol/L methotrexate. (Final magnification ×307.)

control group with Fisher's least significant difference method (one-tailed test) and were considered significant at $p < 0.05$.

Results

Cytologic-nuclear analyses. Exposure of monolayers of BeWo cells to culture media containing forskolin or methotrexate for 48 hours resulted in reproducible morphologic changes similar to those that had been previously described for the latter agent.[10, 19] In serum-free medium, relative to untreated control cells (Fig. 1, *A*), both cytoplasmic and nuclear compartments of the cells were noted to enlarge after 48 hours of incubation with either forskolin or methotrexate (Fig. 1, *B* and *C*, respectively.) In addition, the nuclei of treated cells developed lobations and prominent nucleoli. Planimetric morphometry of the nuclear areas of fixed BeWo cells revealed 3.0- and 1.8-fold increases after exposure to forskolin and methotrexate, respectively, which differed significantly from the mean area of control nuclei ($p < 0.05$). These increases in nuclear size were associated with increases in DNA synthesis (1.7- and 1.8-fold, respectively) as determined by [^{3}H]thymidine incorporation. However, despite increases in DNA synthesis, the treatments did not appear to stimulate cell division relative to the control condition (Table I).

Hormone activities. To assess the endocrine activities of these cells, production of the quintessential protein and steroid hormones of human placental origin, hCG and estrogen, was quantified. Radioimmunoassays of conditioned media demonstrated that hCG-β secretion was increased over basal concentrations by forskolin (7.3-fold) and methotrexate (2.3-fold) (Table II). Dose-response relationships of hCG-β secretion to increasing concentrations of forskolin revealed that 10 μmol/L was optimal in promoting this differentiated response. It was also noted that the stimulation of [^{3}H]thymidine incorporation appeared to be coupled to the induction of hCG-β secretion by forskolin, in a parallel dose-dependent manner (Fig. 2).

Similarly, activity of the rate-limiting enzyme system responsible for placental estrogen production, aromatase, was augmented in the presence of the two agents. Methotrexate (1 μmol/L) induced a 4.9-fold stimulation of basal BeWo aromatase activity, whereas forskolin (10 μmol/L) increased the conversion of an-

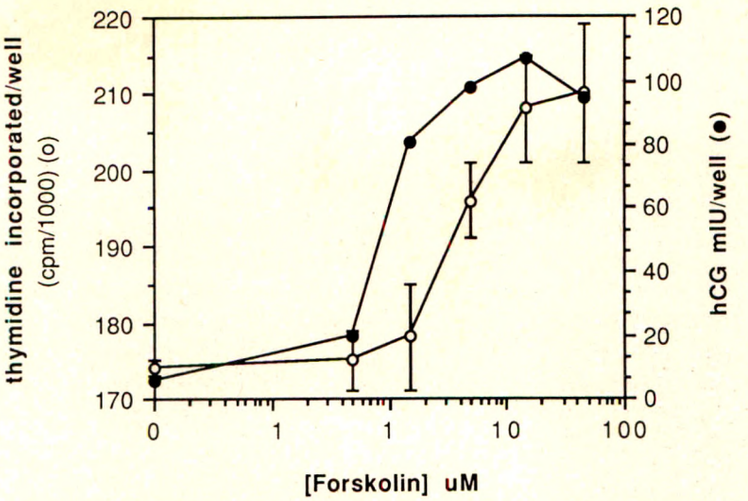


Fig. 2. Correlation of [^{3}H]thymidine incorporation and hCG-β secretion in BeWo cells exposed to forskolin. Increasing concentrations of forskolin were added to cultures of BeWo cells grown in 24-well dishes. Cellular incorporation of [^{3}H]thymidine *(open symbols)* and hCG-β secretion into the culture media *(closed symbols)* were determined after 48 hours as described in material and methods. The *error bars* represent the SEM of triplicate determinations.

Table I. Effects of treatment of cytologic-nuclear changes

Treatment	*Cell number ($\times 10^5$)*	*Nuclear area (μm^2)*	*[^{3}H]thymidine incorporation (cpm/cell)*
Control	2.0 ± 0.5	300 ± 20	0.9 ± 0.2
10 μmol/L Forskolin	2.9 ± 0.3	904 ± 66*	1.5 ± 0.1
1 μmol/L Methotrexate	2.1 ± 0.2	539 ± 46*	1.6 ± 0.3*
Analysis of variance	NS	$p < 0.05$	$p < 0.05$

*Values are significantly greater than control by Fisher's multiple comparison method ($p < 0.05$).

Table II. Effects of treatment on endocrine changes

Treatment	*hCG-β secretion (mIU/ml/10^5 cells)*	*Aromatase activity (pmol/hr/10^5 cells)*	*cAMP content (fmol/10^5 cells)*
Control	13 ± 3	0.5 ± 0.1	65 ± 3
10 μmol/L Forskolin	95 ± 17*	6.9 ± 1.0*	7670 ± 58*
1 μmol/L Methotrexate	30 ± 7	2.5 ± 0.1*	45 ± 2
Analysis of variance	$p < 0.05$	$p < 0.05$	$p < 0.05$

*Values are significantly greater than control by Fisher's multiple comparison method ($p < 0.05$).

drogen precursor to estrogen by a factor of 13.5 (Table II). The combination of forskolin and methotrexate at these doses afforded additive and, in some cases, synergistic effects on aromatase activity, suggesting that the two agents activate different intracellular pathways (data not shown).

Forskolin is a potent activator of adenylyl cyclase.[20] To test whether the effects of this agent on the biochemical cytodifferentiation of BeWo cells was mediated via cAMP, similar experiments were carried out with the use of 1 mmol/L dibutyryl cAMP. After 48 hours, aromatase activity in the presence of dibutyryl cAMP was increased 3.2-fold over control levels, corroborating the results of others.[5] We investigated if the similar phenotype induced by methotrexate might be mediated by an increase in cellular cAMP levels. Using a specific cAMP radioimmunoassay, we were unable to detect an effect of methotrexate on the accumulation of this "second messenger." As expected, forskolin stimulated a large increase (>100-fold) in BeWo cell cAMP content (Table II).

Endocrine gene expression. Consonant with the stimulation of their detectable protein activities, increases in the steady-state levels of the mRNAs encoded by the hCG-β and human aromatase genes were also demonstrated in BeWo cells. By Northern blot analyses,

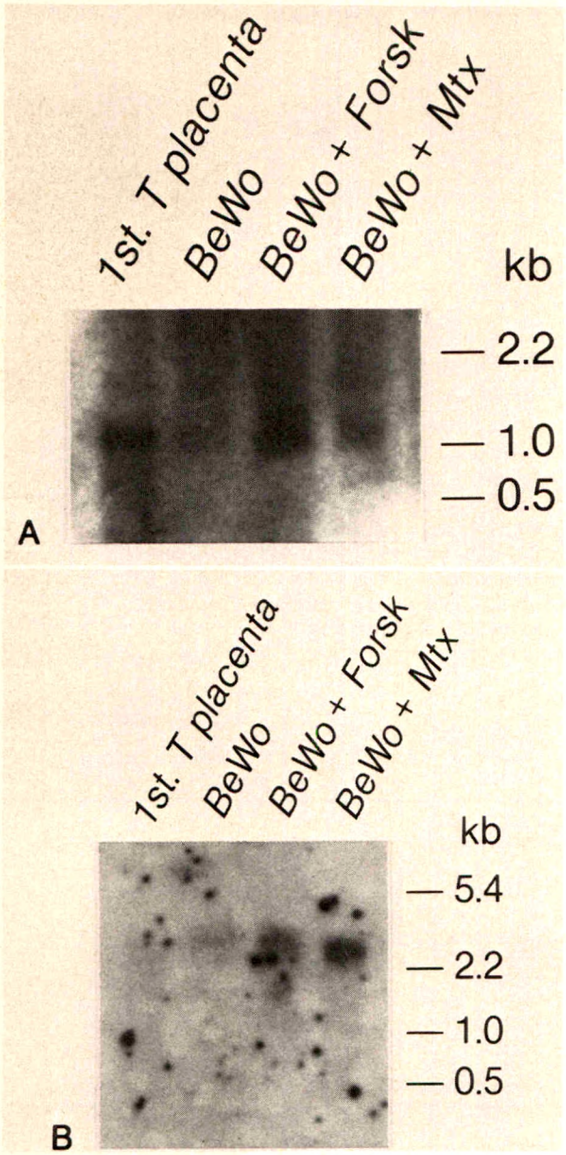


Fig. 3. Northern blot analyses of hCG-β and aromatase mRNA levels in BeWo cell cultures. **A,** Total RNA (10 μg per lane) from control cells *(BeWo)* and cells stimulated with forskolin *(BeWo + Forsk)* or methotrexate *(BeWo + Mtx)* were electrophoresed, blotted, and probed with [^{32}P]cDNA specific for hCG-β. Ten micrograms of normal, first-trimester human placental RNA *(1st. T placenta)* served as a positive control. A representative autoradiogram, revealing the characteristic 1.1 kb transcripts, is shown. **B,** The same Northern blot described in **A** was stripped and hybridized with a [^{32}P]cDNA probe specific for the human aromatase gene. The autoradiogram demonstrated dominant 2.9 kb transcripts. In contrast to the level of expression of hCG (see preceding text), normal, first-trimester placenta did not express significant levels of aromatase mRNA.

a 1.1 kb mRNA species complementary to the hCG-β gene was detected in total BeWo cell RNA. Intact, first-trimester placental tissue, in which high levels of this mRNA are expressed, served as a positive control. Quantification by laser densitometry showed that forskolin increased the concentration of hCG-β mRNA 4-fold, whereas methotrexate stimulated the accumulation of this message by 2.5-fold over basal levels (Fig. 3, *A*). When the Northern blots were probed for human aromatase mRNA expression, a dominant 2.9 kb species was detected. BeWo cells exposed to forskolin and methotrexate expressed this mRNA at concentrations 5- and 7-fold, respectively, over basal levels in unstimulated BeWo cells (Fig. 3, *B*). As expected from the endocrine physiology of the first-trimester placenta, in contrast to its prominent expression of hCG mRNA, levels of aromatase gene expression were barely detectable in this tissue.

Comment

Our findings demonstrate that two chemical agents, very different in structure and known mechanisms, both induce similar changes in the phenotype of cultured human trophoblast cells. BeWo cell stimulation with either forskolin or methotrexate resulted in morphologic and biochemical transformation, which is reminiscent of trophoblast differentiation in vivo. Two observations suggested that these agents operate through independent intracellular pathways. Forskolin is known to activate adenylyl cyclase by binding to the activated complex of its catalytic subunit and the G_s (guanine nucleotide stimulatory) protein.[20] As we have shown, BeWo cells responded to this agent with a dramatic increase in intracellular cAMP levels. Moreover, we and other investigators[5] have documented that cAMP analogs can stimulate estrogen biosynthesis in trophoblast cells, albeit to a lesser extent than forskolin. Activation of cAMP-dependent pathways also increases hCG expression in trophoblast cells.[5-7] Methotrexate, an antimetabolite and inhibitor of reduced folate coenzymes,[21] has complex pharmacologic properties but under the conditions of our studies did not stimulate intracellular cAMP levels. Furthermore, addition experiments using maximally effective doses of forskolin and methotrexate led to additive or synergistic responses in aromatase activity, suggesting that different pathways are involved. Nonetheless, cAMP-independent effects of forskolin, including inhibition of membrane glucose transporters and modulation of voltage-dependent potassium channels, have been documented.[20] Further characterization of these pathways is ongoing to ascertain whether methotrexate and forskolin might share signalling networks in trophoblast cells.

Studies in cultured choriocarcinoma cells have previously documented the effects of cAMP on levels of hCG protein,[5, 6] mRNA,[7, 22, 23] and aromatase activity.[5] In the studies of Lobo et al.,[24] using trophoblast cultures from normal term placenta, dibutyryl cAMP stimulated hCG secretion but not aromatase activity. Ringler et al.[7] also demonstrated subtle differences between the in vitro responses of normal and malignant trophoblast

cells, cautioning that models such as that reported here may not be directly applicable to the process of normal cytotrophoblast differentiation. Nevertheless, the coordinated augmentation of hCG-β and aromatase gene expression in our experiments, as well as its association with cytologic and nuclear differentiation, suggests that the morphologic and biochemical characters of trophoblast may be coupled as described in normal and malignant placental tissue in vivo.[1, 4, 25]

One unexpected observation in these studies was that a single aromatase mRNA transcript of 2.9 kb was detected in the BeWo cells. Previous studies using human term placentas had demonstrated the existence of two aromatase mRNA species (2.5 and 2.9 kb), which are derived from differential processing at alternative polyadenylation sites.[26] Whether the current findings represent preferential expression of the longer transcript in BeWo cells or failure to resolve the two mRNAs is under investigation.

Our experiments demonstrated that forskolin and methotrexate can induce BeWo choriocarcinoma cells in vitro to recapitulate biochemical and morphologic changes consistent with those noted in vivo during normal trophoblast differentiation. These studies extend previous investigations by confirming that two dissimilar xenobiotic agents have similar effects on trophoblast phenotype. That both agents induce a common phenotype supports the hypothesis that intermediate trophoblast represents a discrete step in placental differentiation, which is likely to be modulated in vivo by a variety of differentiation and growth factors. Moreover, our results indicated that the stimulation of hCG-β and aromatase gene expression by these agents was mediated at a pretranslational level. This observation is consistent with the nuclear run-on data of Jameson et al.,[27] which demonstrated that 8-bromo-cAMP enhances the rate of hCG gene transcription in choriocarcinoma cells. The precise mechanisms and pathways activated by forskolin and methotrexate in our BeWo cell model are currently unknown.

Other in vitro models of trophoblast cytodifferentiation[28, 29] suggest that isolated cytotrophoblasts from normal term placenta fuse to generate functional and morphologic syncytia. Recent studies, using isolated normal trophoblasts under serum-free conditions, have documented the development of mature endocrine function in the absence of true syncytium formation.[30] Our model, similar to the latter report, appears to be representative of an intermediate stage of trophoblast cytodifferentiation. In the presence of forskolin or methotrexate, small cytotrophoblast-like BeWo cells enlarged, developed a vacuolated cytoplasm, nuclear swelling, and lobation, and increased prominence of the nucleoli. Within the short time course of our experiments (48 hours), fusion to form true syncytia was not observed. These morphologic findings, as well as the acquisition of mature endocrine functions, are typical of intermediate trophoblast as characterized by Kurman et al.[3] This transitional form of trophoblast cell is believed to be the proximate precursor of the fully functional hormone-producing syncytium. In addition, intermediate trophoblast is thought to play an important role in implantation and the formation of the uteroplacental circulation.[4] We believe that our model system will provide a useful in vitro system to investigate this critical stage of human trophoblast differentiation.

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Factors influencing the initiation of nonshivering thermogenesis

Tania R. Gunn,[a] Karen T. Ball, MSc,[a] Gordon G. Power, MD,[b] and Peter D. Gluckman[a]

Auckland, New Zealand, and Loma Linda, California

The aim of this study was to observe the sequential effects of oxygenation, umbilical cord occlusion, and finally cooling on circulating catecholamines and nonshivering thermogenesis in fetal sheep. We studied five fetal sheep at 132 ± 3 days' gestation. The fetuses were first ventilated with oxygen; Pao_2 levels were maintained above 150 mm Hg, and by 60 minutes there was a significant rise in both plasma glycerol and free fatty acid levels. After umbilical cord occlusion there was a peak in plasma catecholamine and triiodothyronine levels but no significant increase in lipolysis. Cooling, by circulating cold water through a coil around the fetus, induced maximum lipolysis and temperature difference between brown fat and body core, when plasma catecholamine levels were falling. During this study the changes in plasma catecholamine levels did not correlate with the onset of nonshivering thermogenesis. Cutaneous cooling, which causes neurally mediated sympathetic stimulation of brown adipose tissue, is the major signal for the initiation of nonshivering thermogenesis and thus neonatal adaptation. (Am J Obstet Gynecol 1991;164:210-7.)

Key words: Cooling, catecholamines, nonshivering thermogenesis

From the Department of Paediatrics, School of Medicine, University of Auckland,[a] and the Division of Perinatal Biology, School of Medicine, Loma Linda University.[b]
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Reprint requests: Dr. T. Gunn, Department of Paediatrics, University of Auckland, Auckland, New Zealand.
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At birth the fetus is rapidly subjected to cooling, increased oxygenation, and separation from placental blood flow; because these events occur virtually simultaneously, it has not been possible technically to study the effects of the individual factors. In this study we sought to observe separately the effects of oxygenation, β-adrenergic stimulation, occlusion of the umbilical

cord, and cooling of the fetus on the initiation of nonshivering thermogenesis.

Prolonged and intensive cooling of the amniotic fluid and fetal sheep in utero does not stimulate nonshivering thermogenesis,[1] although shivering does occur[2] and fetal plasma catecholamine concentrations rise significantly.[3] Oxygenation of the cooled fetal sheep in utero by ventilation has been shown to stimulate moderate nonshivering thermogenesis, whereas umbilical cord occlusion caused a further rapid increase in fetal plasma glycerol and free fatty acid concentrations.[4]

Significant increases in plasma catecholamine concentrations occur at birth in the human infant and lamb.[5,6] The catecholamine surge induced by umbilical cord cutting has been postulated to be the factor triggering nonshivering thermogenesis at birth.[7] The aim of this study was to evaluate the relative role of a rise in plasma catecholamine levels and of the reflex stimulation of the sympathetic nervous system in the initiation of thermogenesis at birth.

Methods

Surgical procedures. Five Romney ewes carrying lambs from 122 to 140 days' gestational age were operated on while they were under halothane-oxygen general anesthesia. Maternal tarsal arterial and venous catheters and fetal carotid arterial and jugular venous catheters were inserted aseptically. Calibrated thermistors were placed in the maternal vena cava and fetal esophagus to record core body temperature. Another thermistor was placed near a fetal kidney to measure perirenal brown fat temperature. A 5 m coil of plastic tubing was placed around the fetal thorax and neck,[8] and a loop of tape was placed loosely around the umbilical cord. A tracheal cannula (6 mm outer diameter) was inserted and connected to a length of tubing (7 mm inner diameter; dead space 15 ml) for later ventilation of the fetus. The breathing tube, thermistor leads, afferent and efferent arms of the coil, snare, and catheters were exteriorized through an incision in the maternal flank.[4] During the recovery period tracheal fluid was allowed to drain into the amniotic fluid. Experiments were carried out the following day, while the ewes stood in a metabolic cage in the laboratory at 20° C and 50% relative humidity. They were given free access to hay, alfalfa, sheep "nuts," and water throughout the study.

Experimental procedures. Five fetuses were studied. Temperature readings (±0.01° C) were recorded at 1-minute intervals throughout the study, and blood samples were collected at 15-minute intervals. The experiment began with a 30-minute control period. Fluid was then drained from the lungs and the tracheal tube was connected into a closed rebreathing circuit. This circuit consisted of a respiratory pump (Harvard model 607), 1 L spirometer, appropriate one-way valving, and a carbon dioxide absorber.[4] The settings were adjusted periodically to maintain arterial $Paco_2$ in the range 40 to 55 mm Hg and Pao_2 above 100 mm Hg.

After 60 minutes of oxygen ventilation an isoproterenol infusion (0.04 to 0.08 μg/kg/min) was commenced and continued for the next 210 minutes. After 60 minutes of this infusion the snare around the umbilical cord was pulled tight. Ventilation and isoproterenol were continued for a further hour, and then cooling was begun. Passing chilled tap water (8° to 19° C) through the coil at a rate of 180 to 280 ml/min caused the fetal core temperature to fall 2° to 3° C.[8]

To complete the experiment the cooling was stopped, and after 30 minutes of spontaneous rewarming the isoproterenol infusion was discontinued and the thermogenic responses were monitored for a further 30 minutes.

After completion of the study the ewe and fetus were put to death with an overdose of barbiturate. The fetus was weighed to the nearest gram, and the location of the thermistors and occlusion of the umbilical cord were verified. The thermistors were recalibrated to establish that their characteristics had not changed during implantation.

Blood sampling. Fetal carotid arterial blood samples (2.1 ml) were collected anaerobically and promptly analyzed for blood gas and pH values. The plasma was then removed after centrifugation and stored at −20° C for later analysis.

Analytic procedures. pH and Po_2 were measured at 39° C with microelectrodes (Radiometer model BMS-3). Observed values were corrected to the actual body temperature of the fetus at each phase of the experiment.[9] Thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3) were measured by radioimmunoassay. All iodothyronine measurements were made in single assays. The within-assay coefficients of variation were 8.9% for T_4 and 7.7% for T_3. All samples had concentrations appreciably above the limit of sensitivity of the assays. Glycerol was analyzed after enzymatic conversion with glycerokinase according to the method of Pinter et al.[10] Plasma free fatty acids were determined by the colorimetric method of Falholt et al.[11] Palmitic acid was used as a standard, and a sample size of 0.1 ml was used to increase the sensitivity. The within-assay and between-assay coefficients of variation were 2.2% and 4.1% for glycerol and 9.0% and 14.1% for free fatty acids, respectively. Norepinephrine and epinephrine were measured by radioenzymatic assay[12] as described previously.[3] Results are provided as means ± SEM. Statistical comparisons were made after preliminary analysis

Table I. Changes in fetal arterial pH, Pa_{O_2}, and core temperature at intervals during the study: Control, ventilation with oxygen, isoproterenol infusion, umbilical cord occlusion, cooling, rewarming, and discontinuation of isoproterenol

	pH	*P_{O_2} (mm Hg)*	*Temperature (° C)*
Control	7.42 ± 0.01	20.8 ± 1.7	39.74 ± 0.11
+ 15 min, ventilation	7.38 ± 0.01	198.2 ± 40.4	39.92 ± 0.16
+ 60 min, ventilation	7.38 ± 0.02	213.4 ± 39.4	40.02 ± 0.22
+ 15 min, isoproterenol	7.37 ± 0.02	183.5 ± 32.9	40.01 ± 0.22
+ 60 min, isoproterenol	7.37 ± 0.03	227.2 ± 29.7	39.92 ± 0.18
+ 15 min, cord snare	7.26 ± 0.05	232.3 ± 21.1	40.20 ± 0.21
+ 60 min, cord snare	7.15 ± 0.07	202.4 ± 38.7	40.99 ± 0.38
+ 15 min, cooling	7.12 ± 0.08	197.9 ± 25.2	39.67 ± 0.60
+ 60 min, cooling	7.08 ± 0.02	151.7 ± 13.7	37.46 ± 0.62
+ 15 min, warming	7.21 ± 0.03	175.0 ± 30.9	38.29 ± 0.51
+ 30 min, warming	7.30 ± 0.01	194.8 ± 31.3	38.81 ± 0.56
+ 15 min, stop isoproterenol	7.39 ± 0.02	257.4 ± 22.6	39.31 ± 0.39
+ 30 min, stop isoproterenol	7.40 ± 0.03	270.6 ± 5.7	39.48 ± 0.33

Means and SEs for five fetal sheep are given.

of variance between the means at the start of each episode of the study and single experimental values at the end of that episode by use of Student's *t* test.

These procedures were approved by the Animal Ethics Committee of the University of Auckland.

Results

The five fetal sheep were 132 ± 3 days' gestation and weighed 3.2 ± 0.3 kg.

Control period. In the control period the fetal core and brown fat temperatures were in the normal fetal range of 39.74° ± 0.11° C, and they were not significantly different. The fetal core temperature averaged 0.21° C higher than the maternal core temperature. The fetal arterial P_{O_2} was 20.8 ± 1.70 mm Hg and pH was 7.42 ± 0.01 (Table I). Plasma T_3 and T_4 levels, glycerol concentration (57.9 ± 9.0 μmol/L), and free fatty acid levels (37 ± 8 μEq/L) were all in the normal fetal range. Plasma norepinephrine and epinephrine concentrations were 358 ± 124 and 293 ± 136 pg/ml, respectively.

Ventilation with oxygen. Five minutes after the start of ventilation the fetal arterial P_{O_2} rose to 198.2 ± 40.4 mm Hg and was maintained above 150 mm Hg for the next 5 hours. There was no significant change in pH (Table I). There were also no significant changes in fetal or maternal temperatures or in plasma catecholamine or thyroid concentrations. After 60 minutes plasma glycerol levels had risen to 105 ± 2.3 μmol/L ($p < 0.02$) (Fig. 1), and plasma free fatty acids had risen to 97 ± 26 μEq/L ($p < 0.02$).

Isoproterenol infusion. The infusion of the β-adrenergic receptor agonist isoproterenol for 60 minutes did not cause any significant change in fetal temperatures, oxygenation, pH, plasma catecholamine levels or thyroid hormone levels. By 60 minutes plasma glycerol values increased to 183 ± 61 μmol/L ($p < 0.05$) (Fig. 1) and plasma free fatty acids increased to 132 ± 33 μEq/L ($p < 0.04$); significance is after comparison with values after 60 minutes of ventilation.

Umbilical cord occlusion. Snaring of the umbilical cord was followed by a 1.08 ± 1.02° C steady rise in fetal core and brown fat temperature to 40.99° C after 60 minutes. The fetal arterial P_{O_2} was 202 ± 87 mm Hg and pH was 7.15 ± 0.07. There was a significant increase in fetal plasma norepinephrine and epinephrine concentrations to a peak of 2553 ± 937 pg/ml ($p < 0.05$) (Fig. 2, *A*) and 2409 ± 990 pg/ml ($p < 0.05$), respectively, after 60 minutes of cord occlusion (Fig. 2, *B*). The plasma T_3 concentrations rose to 1.14 ± 0.31 nmol/L ($p < 0.01$) whereas the plasma T_4 levels were unchanged (Fig. 3). The plasma glycerol concentration was 286 ± 81 μmol/L, and the free fatty acid level was 194 ± 35 μEq/L (not significant compared with the previous period).

Cold exposure. Fetal core temperature fell 3.53° ± 0.56° C after 60 minutes of cooling while brown fat temperature fell 2.54° ± 0.56° C during this period. The fetal brown fat minus core temperature difference had increased to 0.99° ± 0.35° C ($p < 0.01$), indicating vigorous nonshivering thermogenesis (Fig. 1). The fetal arterial P_{O_2} was 151.7 ± 30.6 mm Hg, and there was an increasing acidosis (pH 7.08 ± 0.04) after 60 minutes of cooling. The plasma norepinephrine and epinephrine concentrations fell during cold exposure ($p < 0.05$) (Fig. 2), whereas there was no change in thyroid hormone levels (Fig. 3) during this period. After 60 minutes of cooling the plasma glycerol levels had risen a further twofold to a peak of 551 ± 190 μmol/L ($p < 0.05$) (Fig. 1) and the free fatty acid concentrations to 313 ± 79 μEq/L ($p < 0.04$).

Warming and discontinuation of isoproterenol. The fetal core and brown fat temperatures returned to control values during this period. The arterial P_{O_2} was 270 ± 13 mm Hg and pH was 7.40 ± 0.03 at the end of the study. The plasma glycerol concentrations fell to 181 ± 72 μmol/L ($p < 0.02$) and free fatty acid levels fell to 87 ± 38 μEq/L ($p < 0.03$) whereas the fetal brown fat minus core temperature decreased rapidly ($p < 0.01$) (Fig. 1). Only the plasma T_3 levels remained

elevated (Fig. 3). Catecholamine levels remained at basal values.

Comment

Effective thermogenesis is essential for successful neonatal adaption to extrauterine life. In the preterm sheep in utero we have studied the effects of sequentially ventilating the fetal lungs with oxygen, infusing a β-adrenergic agonist, occluding the umbilical cord, and finally cooling the fetus. The order in which these events are applied to the fetus is important. In our previous studies we introduced the stimuli in a different order, commencing with cooling, followed by oxygenation and finally umbilical cord snare.[4, 13] No effect of cooling alone on lipolysis could be demonstrated. We found a modest increase in plasma free fatty acid and glycerol levels after oxygenation and a marked increase in these levels after umbilical cord snare. There was a lack of a thermogenic response to cooling alone, although fetal plasma catecholamine concentrations and blood flow to the fetal brown fat increased significantly.[3, 14]

In this study in which the sequence of events was altered, ventilation with oxygen in the absence of cooling caused a small but significant increase in plasma glycerol and free fatty acid concentrations, and infusion of the β-adrenergic receptor agonist isoproterenol caused a further modest rise. The lack of an α_1-adrenergic action by isoproterenol may explain this modest response of plasma glycerol and free fatty acids as there appears to be a synergistic interaction between β-adrenergic and α-adrenergic receptor stimulation during thermogenesis.[15, 16] Alternatively, those agonists circulating in the blood may be appreciably less effective than those released from nerve endings in brown adipose tissue.

Umbilical cord occlusion was followed by only a small further rise in plasma glycerol and free fatty acid levels despite a very marked surge in circulating levels of norepinephrine and epinephrine, both of which are mixed β- and α-adrenergic agonists.[17] Recently attention has focused on circulating catecholamines as the dominant signal for the onset of lipolysis at birth. Padbury et al.[7, 18] demonstrated in acutely exteriorized fetal sheep that circulating plasma catecholamine levels rose substantially after umbilical cord cutting, in association with a rise in plasma free fatty acid levels. A greater sympathoadrenal response was found in preterm sheep than in term sheep, with a delayed but exaggerated elevation of both plasma norepinephrine and epinephrine levels but blunted free fatty acid responses.[17] In our study of preterm fetal sheep in utero, where the various events of parturition can be independently evaluated, the timing and levels of peak norepinephrine plasma concentrations after umbilical cord occlusion

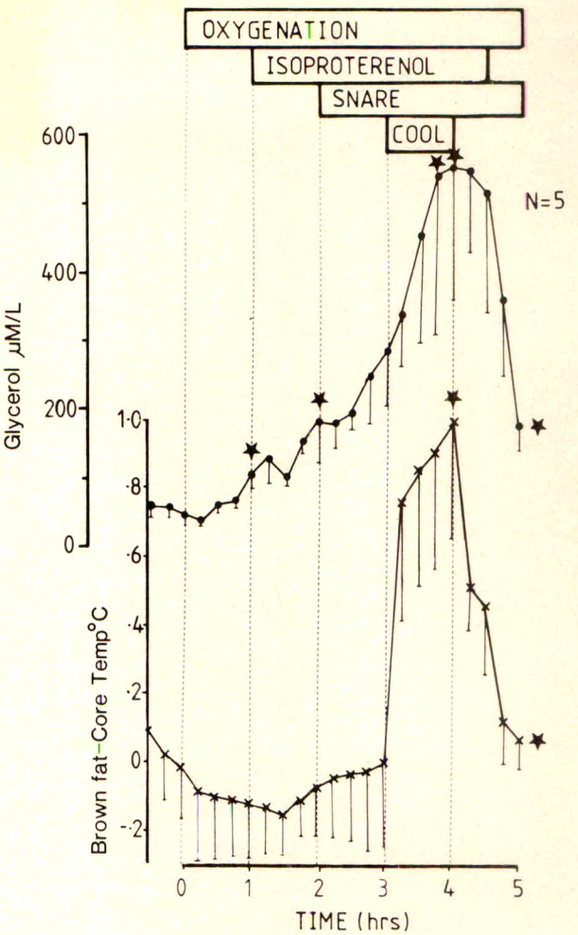


Fig. 1. Time course and responses of plasma glycerol concentrations and fetal brown fat minus core temperature is shown after ventilation with oxygen, infusion of isoproterenol, occlusion of the umbilical cord, cooling, and then rewarming. Means and SEs for five fetal sheep are given. *Star*, $p < 0.05$.

were similar to those found by Padbury et al.[18] after umbilical cord cutting whereas the peak plasma epinephrine concentration was lower and earlier. However, the values achieved were in excess of those suggested to be sufficient to initiate physiologic responses.[19] This study therefore strongly suggests that changes in circulating catecholamine levels are not causal in the initiation of nonshivering thermogenesis because the surge in plasma catecholamines did not correlate with changes in indices of thermogenesis and points to other factors as major determinants of nonshivering thermogenesis at birth.

Cooling after cord snare rapidly induced marked lipolysis with a noticeable increase in the fetal brown fat minus core temperature differential. This occurred while catecholamines were decreasing, whereas earlier in the experiment, when catecholamines peaked, thermogenesis was not maximal. The most likely explana-

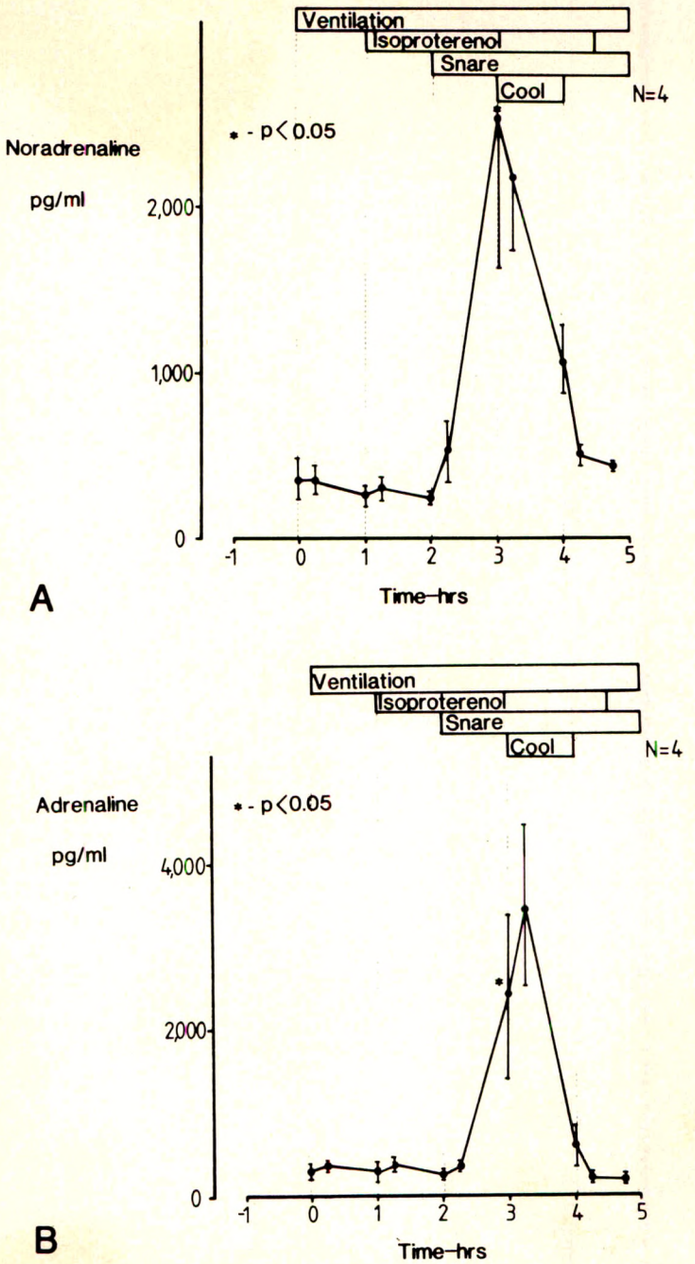


Fig. 2. A, Time course and responses of plasma norepinephrine concentrations are shown after ventilation, isoproterenol infusion, umbilical cord occlusion, cooling, and rewarming. Means and SEs for five fetal sheep are given. **B**, Time course and responses of plasma epinephrine concentrations are shown after ventilation, isoproterenol infusion, umbilical cord occlusion, cooling, and rewarming. Means and SEs for five fetal sheep are given.

tion for this is that cooling induced an increased drive via the sympathetic nervous system leading to noradrenergic stimulation of lipolysis via direct innervation of brown fat. Stimulation of the sympathetic nervous system by cold exposure markedly increases norepinephrine turnover in brown adipose tissues,[20, 21] which may not be reflected by a rise in circulating catecholamine levels. Indeed the role of nervous rather than endocrine regulation of brown fat is supported by studies in the newborn lamb. Agata et al.[22] demonstrated that the majority of circulating norepinephrine in the lamb at birth arises from postganglionic sympathetic neurons. In a previous study we found that changes in plasma catecholamine levels did not parallel changes in

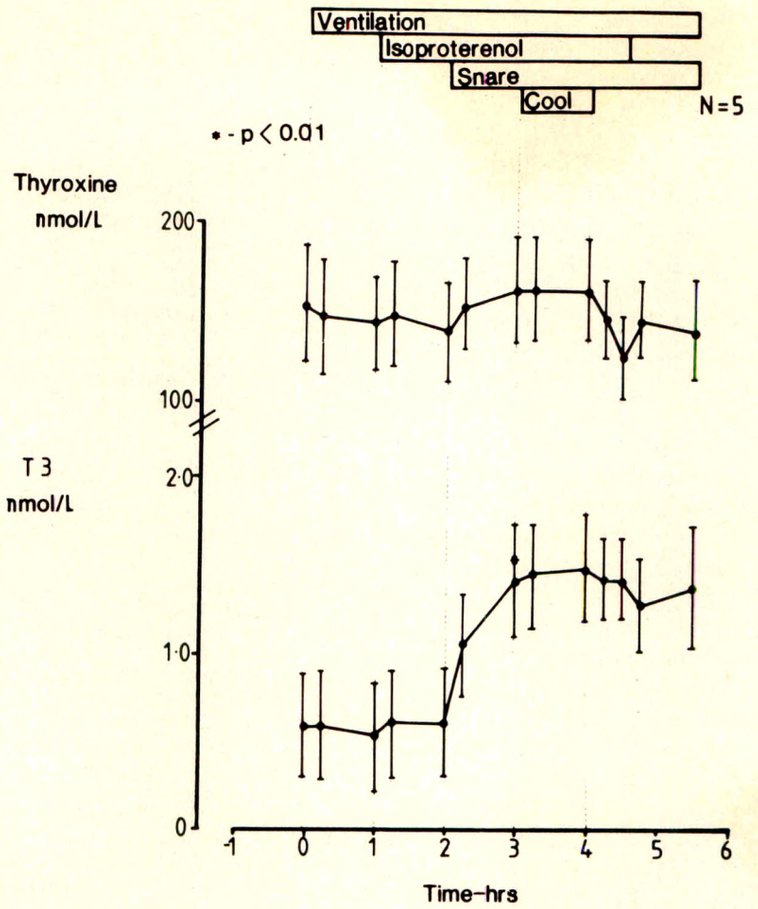


Fig. 3. Responses of plasma thyroxine and T_3 are shown after ventilation, isoproterenol infusion, umbilical cord occlusion, cooling, and rewarming. Means and SEs for five preterm fetal sheep are given.

thermogenic indices.[13] Further adrenalectomy in infant lambs reduced summit metabolism after cold exposure; this was restored by cortisol to 85% of the response in intact animals, and the influence of circulating catecholamines appeared to be small.[23] Such neurally mediated, local control of brown fat agrees with observations that β-adrenergic and ganglionic blockade inhibits ≥80% of nonshivering thermogenic responses in the fetal sheep.[24] Thus it seems clear that sympathetic nerve activity is both necessary and able to explain the onset and control of nonshivering thermogenesis in the fetal sheep.

Removal of the placental circulation by cord occlusion appears to act passively and allows cooling-induced sympathetic activity in brown fat to initiate heat production. Recent evidence suggests that the passive effect of placental separation is due to removal of placental factors that enter the fetal circulation and inhibit nonshivering thermogenesis.[4] The presence of such inhibitors seems to be of benefit to successful neonatal adaptation. If the marked increases in fetal circulating catecholamine levels that have been shown to follow hypoxia[25] and labor[26] induced nonshivering thermogenesis in utero, the increased oxygen demand and heat production would seriously compromise the metabolic state of the fetus. The concentrations of these inhibitory factors fall after cord occlusion, thereby permitting thermogenesis to begin when cooling stimulates sympathetic nerve activity. This study indicates that such an inhibitor might act by blocking sympathetic stimulation of brown fat but it does not identify the site of action.

The euthyroid state in late gestation is necessary for effective thermogenesis. Lambs that underwent thyroidectomy in midgestation become profoundly hypothermic and die after delivery[27]; the serum free fatty acid levels are reduced despite elevated plasma epinephrine concentrations.[28] However, thyroidectomy performed during delivery but before the umbilical cord is severed does not impair the increase in oxygen consumption although the postnatal surge in plasma T_3 is arrested.[29] A thyrotropin surge occurs soon after

birth in human infants,[30] and this also occurs rapidly after the fetal sheep is cooled in utero.[31] The only intervention that caused a significant rise in plasma T_3 concentrations in our study was umbilical cord snare; cooling caused no further increase. A rise in plasma T_3 after cord cutting or snare has been reported.[4, 32] This rise in plasma T_3 may be due to removal of placental inner ring monodeiodinase activity,[33] with an increase in T_4 to T_3 conversion in brown adipose tissue.[34] In our study the failure of circulating T_3 to rise further after cooling was begun and nonshivering thermogenesis was activated speaks against brown fat as a major site of origin of circulating T_3 in the lamb. In brown adipose tissue type II 5′-deiodinase activation is induced by cold exposure,[34, 35] and this enzyme increases intracellular conversion of T_4 to T_3. In rats this local production of T_3 defines the rate of synthesis of uncoupling protein (thermogenin) in brown adipocytes and thus promotes nonshivering thermogenesis.[34] Full expression of the uncoupling protein gene in the brown adipocytes of the rat, in which nonshivering thermogenesis is induced over some days after birth, requires both norepinephrine and T_3. However, in both sheep and man, where nonshivering thermogenesis is initiated immediately at birth, brown fat may be recruited before parturition. This may be induced in part by the gradual rise of T_3 in fetal lambs in late gestation.[36] This would explain the effect of fetal, but not neonatal, thyroidectomy on the initiation of nonshivering thermogenesis. Further, thermogenin messenger ribonucleic acid can be demonstrated in brown fat in late gestation in the bovine and other species with immediate onset of nonshivering thermogenesis.[37]

In conclusion, in the present study plasma free fatty acid and glycerol levels rose to values 10 times greater than control concentrations and to about 80% of the levels achieved in cold stressed newborn lambs.[38] These were comparable to those in prematurely delivered lambs, which have lower rates of nonshivering thermogenesis than term lambs.[39]

Many factors are important for the initiation of effective nonshivering thermogenesis at birth; these include cutaneous cooling, oxygenation, separation from the placenta, and the euthyroid state. The surge of circulating catecholamines after umbilical cord occlusion is not the major signal. Stimulation of the sympathetic nervous system plays a dominant role in the initiation of thermogenesis in response to cold cutaneous receptor stimulation.

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Transplantation of fetal cells

Timothy M. Crombleholme, MD, Jacob C. Langer, MD, Michael R. Harrison, MD, and Esmail D. Zanjani, PhD

San Francisco, California, and Reno, Nevada

Cellular transplantation is an attractive alternative to whole-organ transplantation when only a discrete function of the organ is deficient. Early fetal donor cells have an advantage because they engraft readily and do not cause graft-versus-host disease. Similarly, the fetus is an ideal recipient of allogeneic fetal cells as it is incapable of rejecting them early in gestation. This review presents the theoretical rationale, recent research advances, and clinical implications for adults with diabetes mellitus and Parkinson's disease; we also describe in utero transplantation of fetal hematopoietic stem cells and hepatocytes for the treatment of inherited hemotologic and hepatic deficiencies, as well as the use of fetal islet cells and dopamine-producing cells to treat postnatal conditions. (Am J Obstet Gynecol 1991;164:218-30.)

Key words: Cellular transplantation, graft-versus-host disease, hematopoietic stem cells

Cellular transplantation has long been considered as a possible alternative to whole-organ transplantation for a variety of tissues. Cellular transplantation has the potential advantages of technical ease, placement of cells into an immunologically or physiologically advantageous location, ability to manipulate the cells in culture before transplantation, and convenience of long-term cryopreservation of viable cells. In experimental systems encouraging results have been seen with transplantation of hematopoietic stem cells, pancreatic islet cells, and hepatocytes. However, except for the relatively recent advances in bone marrow transplantation, cellular transplantation has not been widely used clinically.

The use of fetal tissue for transplantation has a number of theoretical advantages. The most important is the potential immunologic "immaturity" of fetal tissue, a concept that arises from the presumption that the recognition of "self" occurs during an early stage of fetal life and that tissue used before that stage would be immunologically privileged. Work in the mouse model has shown that H-2 antigens are expressed at 11 days' gestation, implying that fetal tissue used before that time would not be rejected.[1] Further evidence to support this contention was reported by Foglia et al.,[2] who found in a rat model that transplants of fetal renal tissue were more readily accepted earlier in gestation than later. In addition, they found in their model that graft survival was worse with hepatic tissue than with gonadal and renal tissue, leading them to the conclusion that rejection of fetal grafts is also organ specific.

There are currently several types of fetal cellular transplantation being investigated experimentally: hematopoietic stem cells, pancreatic islet cells, dopamine-containing neuronal cells, and hepatocytes. Although the functional and immunologic advantages of fetal cells have been useful experimentally and clinically in the treatment of adult patients with hematologic, endocrine, metabolic, and neurologic disorders, fetal cellular transplantation also has been used recently in the treatment of these deficiencies in utero.

Fetal hematopoietic stem cell transplantation

Postnatal allogeneic bone marrow hematopoietic stem cell transplantation has been used to successfully treat a broad spectrum of congenital hematologic diseases (Table I). In most cases, however, donors with identical human leukocyte antigen are not available, precluding transplantation. In addition, by the time most of these children are considered for transplantation, they have already been compromised by disease. Recurrent infections, growth retardation, and sensitization by multiple blood transfusions can reduce the likelihood of a successful outcome. Finally, such allogeneic transplants require recipient bone marrow–conditioning regimens that include radiation or chemotherapy with the attendant risks of infection and

From the Fetal Treatment Program, Department of Surgery, University of California, San Francisco, and the Veterans Administration Medical Center, Reno.
Reprint requests: Michael R. Harrison, MD, The Fetal Treatment Program, University of California, San Francisco, 3rd and Parnassus, HSE 585, San Francisco CA 94143-0570.
6/1/19059

failure of engraftment. Graft rejection and graft-versus-host disease are constant threats that require long-term immunosuppression. The combination of the disease and its treatment result in high morbidity and mortality for the pediatric recipient.[3-5]

The transplantation of preimmune fetal hematopoietic stem cells into a preimmunocompetent fetal recipient may allow the creation of a permanent hematochimera without the risks of marrow ablation, rejection, and graft-versus-host disease seen in postnatal bone marrow transplantation. Even partial expression of normal hematopoietic stem cells may be enough to ameliorate or eliminate the clinical manifestations of defective cells.[6] For individuals with inherited defects in bone marrow–produced cell lines that can be diagnosed by chorionic villus sampling (e.g., hemoglobinopathies, immunodeficiencies),[7] reconstitution with a normal cell line early in gestation has many theoretical advantages and considerable clinical appeal.[8]

Experimental work

Some animal research has been done, but many questions remain. The work of Seller and Polani[9] demonstrating experimental chimerism in the mouse has been criticized by others[10] because the histocompatibility differences between the donor and recipient were not clearly established. The work of Fleishman and Mintz[11] is provocative, but others have had difficulty reproducing these results, primarily because of problems with injecting a cell preparation into the placenta of a fetal mouse. The investigation of methods to minimize graft-versus-host disease (by depleting donor material of immunocompetent lymphocytes by density gradient perfusion,[12] agglutination on columns,[13, 14] or treatment with monoclonal antibodies[4, 15]) has been hampered because graft-versus-host disease in the mouse model is not as severe and it is not the same disease as in the human; neither Seller and Polani[9] nor Fleishman and Mintz[11] reported graft-versus-host as a complication from allogeneic fetal hematopoietic stem cell transplants in their studies of mice.

In utero hematopoietic stem cell transplantation in sheep

Our goal in this study was to produce an animal model of hematopoietic chimerism by the in utero transplantation of fetal hematopoietic stem cells. We chose the sheep for the following reasons: (1) The sheep has two naturally occurring alleles at the β-hemoglobin locus (A and B) that can be used as definitive markers of successful engraftment and chimerism; (2) gestation in the pregnant ewe is sufficiently long (term = 145 days) to allow experimental manipulation in the first trimester; (3) the immune status of the fetal lamb is relatively well documented, permitting accurate selection of preimmune donors and recipients. We transplanted hematopoietic stem cells obtained from the combined livers of 35- to 50-day-old fetal AA sheep and 45- to 65-day-old homozygous BB fetuses via the intraperitoneal route (Fig. 1). Fetal liver hematopoietic cell suspensions were prepared and injected (2 to 5 × 10^8 cells per kilogram estimated fetal weight) into the peritoneal cavity of fetuses exposed by the amniotic bubble technique (Fig. 2).

Table I. Congenital hematologic diseases successfully treated by postnatal bone marrow transplantation and potentially amenable to in utero hematopoietic stem cell transplantation

β-Thalassemia[3]
Sickle cell anemia[4]
Wiskott-Aldrich syndrome[5]
Chronic granulomatous disease[7, 8]
Kostman's syndrome[10]
Infantile malignant osteopetrosis[11]
Chediak-Higashi syndrome[12]
Maroteaux-Lamy syndrome[13]
Severe combined immunodeficiency syndrome[14]

As with all surgical procedures on the fetus early in gestation, there was a high perioperative mortality. Four fetuses survived the procedure. Of these, three were born with clear evidence of donor cell engraftment as determined by both hemoglobin (Table II) and chromosome (Table III) analysis. This study represented the first successful creation of experimental chimerism in a large animal model, without the use of immunosuppression and without the development of graft-versus-host disease.

We then documented that the donor stem cells primarily engraft in the host marrow and undergo proliferation without significant differentiation until the time during ontogeny when marrow assumes an important role in hematopoiesis.[16] Donor cells obtained from livers of twin 120-day-old fetal sheep homozygous for type A hemoglobin were injected into preimmune (55 to 62 days old) type B fetuses intraperitoneally (6×10^6 nucleated cells per kilogram), and bone marrow, liver, and peripheral blood cells of hosts were analyzed for presence of donor hemoglobin and hematopoietic progenitors at intervals after transplant. Nine of 14 surviving fetuses-lambs exhibited significant donor cell engraftment. About 91% of all donor stem cells detectable by in vitro clonal assays were present in host marrow. At 15 days after transplant, donor erythroid progenitors constituted about 12% of all host marrow colony-forming units, erythrocyte–burst-forming units, erythroid. Although relative percentage of donor colony-forming units, erythrocyte–burst-forming units, erythroid remained unchanged at days

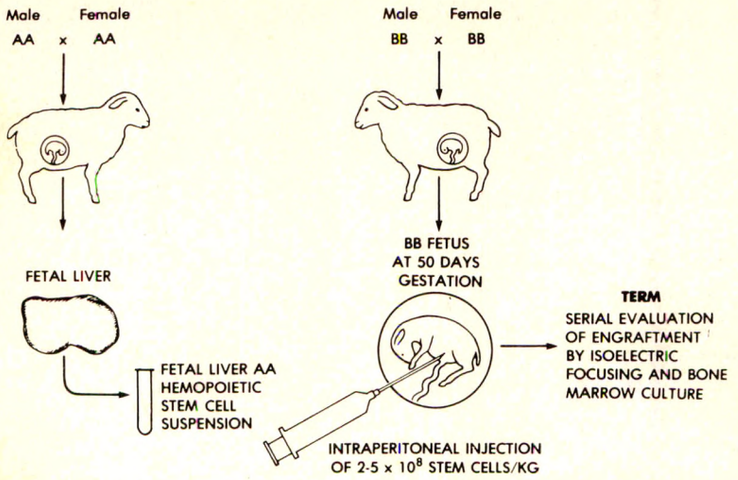


Fig. 1. The sheep model for in utero hematopoietic stem cell transplantation used a naturally occurring polymorphism at the β-hemoglobin locus. Fetal livers were harvested as a source of hematopoietic stem cells from sheep with pure type AA β-hemoglobin. These type AA hematopoietic stem cells were then injected intraperitoneally into a recipient fetus of β-hemoglobin phenotype BB. This polymorphism allowed identification of donor hemoglobin in the postnatal recipients by hemoglobin electrophoresis.

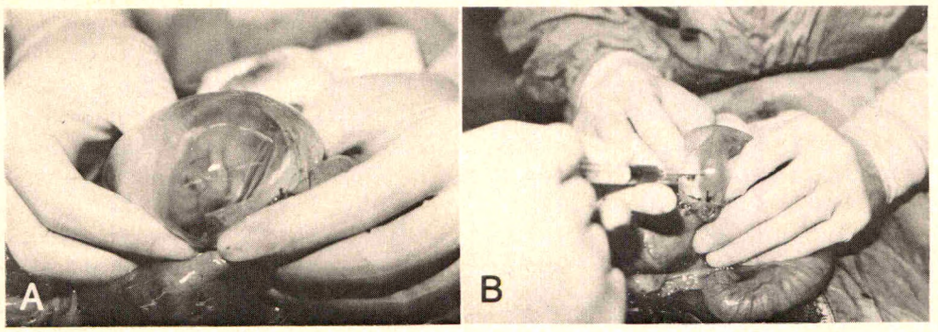


Fig. 2. A, The microbubble technique was used to perform hematopoietic stem cell transplants. The myometrial layers are divided with electrocautery and care is taken to preserve the amnion intact. The fetus can then be manipulated under direct vision. **B,** Injection of hematopoietic stem cells into peritoneal cavity of fetal sheep recipient is easily accomplished.

40 and 70 after transplantation and at 1 month after birth, their absolute numbers in marrow (calculated per femur) increased by 30%, 85%, and 420%, respectively. No donor hemoglobin was detected in host blood on days 15 and 40 after transplantation. Donor hemoglobin in host blood was first detected at day 70 after transplant, close to the time when marrow becomes a major blood cell–producing tissue in sheep. Both at birth and at 1 month after birth, donor hemoglobin constituted about 11% of total blood hemoglobin (Fig. 3).

Our results in the allogeneic hematopoietic stem cell transplant experiments demonstrate that a significant portion of the recipient fetuses exhibited sustained engraftment of allogeneic donor cells, providing support for the concept that the fetus is an ideal recipient for hematopoietic stem cell transplantation. The absence of graft-versus-host disease in long-term chimeric lambs suggests that the preimmune animal is also the ideal stem cell donor.

Fetal hematopoietic stem cells as a vehicle for gene transfer

W. French Anderson and collaborators at the National Institutes of Health have been developing gene therapy as another potential mode of treatment for genetic disease.[17] They have shown that retroviral vectors designed to carry heterologous genes are effective in delivering functional genes into many cell types in vitro[18, 19] and into mouse hematopoietic cells in vitro.[20, 21] We are participating in a study on gene transfer in the lamb that further illustrates that the fe-

Table II. Relative distribution of hemoglobins A (donor) and B (recipient) in lambs transplanted in utero

Animal no.	*Hemoglobin type**	*Hemoglobin (%)*					
		At 1 wk	*At 1 mo*	*At 2 mo*	*At 3 mo*	*At 4 mo*	*At 6 mo*
1276	A (donor)	0	0	0	0	0	—
	B (host)	100	100	100	100	100	—
558†	A (donor)	26	—	—	—	—	—
	B (host)	74	—	—	—	—	—
1286	A (donor)	21	29	17	22	24	23
	B (host)	79	71	83	78	76	77
1840	A (donor)	16	18	20	17	14	14
	B (host)	84	82	80	83	86	86

*Hemoglobin types were determined on cold peripheral red blood cell lysates by isoelectric focusing. Percentage of the two hemoglobin types was determined on reticulocyte-enriched triatiated leucine–labeled peripheral blood lysates or, when required, leucine-labeled bone marrow aspirates by chromatography on carboxymethylcellulose columns.

†Animal died of sepsis at 1 month of age.

Table III. Gestational age of recipient fetuses at transplantation, phenotypic sex, and karyotype of newborn lambs at 1 week of age*

Animal no.	*Gestational age (days)*	*Phenotype*	*XY (%)*	*XX (%)*
1287	62	Female	0	100
553	55	Male	90	10
1286	62	Female	11	89
1340	55	Male	88	12

From Flake AW, Harrison MR, Adzick NS, Zanjani ED. Transplantation of fetal hematopoietic stem cells in utero: the creation of hematopoietic chimeras. Science 1986; 233:776-8. Copyright 1986 by the American Association for the Advancement of Science.

*Chromosomal analyses were performed on peripheral blood lymphocytes, as well as cultured bone marrow cells. Results from bone marrow are presented here.

tus may be an ideal donor-recipient of hematopoietic stem cell transplants.[22]

To test the feasibility of fetal hematopoietic stem cell–gene transfer, an in utero sheep gene transfer protocol was established. Circulating hematopoietic cells from a 100-day-old fetal lamb were removed by exchange transfusion, injected with N2 vector by the supernatant infection protocol, and reinfused back into the lamb. The fetal lamb was allowed to grow in utero and was born normally at term (145 days' gestation). One week after birth, bone marrow cells from the lamb were evaluated in colony assays in the presence or absence of G418. However, G418 colonies were obtained in the infected animal (colony-forming units, culture = 22%; colony-forming units, mixed = 39%; burst-forming units, erythroid = 35%; colony-forming units, erythrocyte = 22%), representing successful expression of G418 resistance 62 days after transplantation. Whether this represents true expression of the Neo gene awaits further studies.

These preliminary experiments demonstrated that fetal sheep stem cells can be infected efficaciously. It was not clear whether this high level is due to species differences or to an inherent quality of fetal cells or to both. To assay whether the high degree of gene transfer in this model was caused by a greater efficiency of Neo transfer into fetal than adult progenitors, we compared the relative rate of Neo into fetal and adult hematopoietic cells of sheep and the human.[23] Equivalent numbers of adult or fetal cells were incubated with N2-containing supernatant overnight, washed, and cultured in clonogenic assay with and without G418 (2 mg/ml). The infected control cells failed to produce hematopoietic colonies in the presence of G418. In contrast, Neo-infected sheep and human cells produced G418-resistant colonies (Table IV). Overall, fetal cells exhibited a significantly higher degree of Neo transfer than adult hematopoietic cells. There also appeared to be species differences as the effciency of gene transfer was higher in the sheep than the human cells. The gene transfer of Neo into human cells could be dramatically augmented, however, by incubation with a mixture of recombinant human interleukin IL-3 and granulocyte-macrophage colony-stimulating factor in addition to the retroviral vector. The number of adult and fetal resistant colonies (except colony-forming units, erythroid) increased by 300% and 184%, respectively. The greater efficiency of exogenous gene transfer into hematopoietic progenitors further supports the fetus as an ideal donor, in this case for in utero gene therapy.

These findings suggest that in utero transplantation–gene transfer may provide a viable adjunct to postnatal gene transfer or even an alternative when diagnosis is

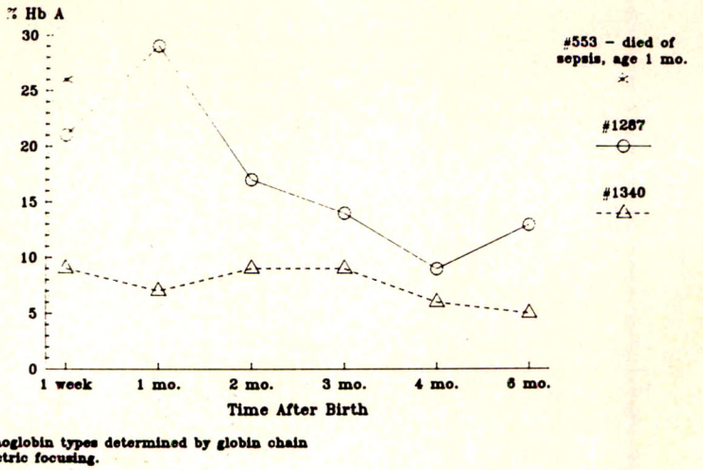


Fig. 3. Two of the three surviving hematopoietic stem cell transplant recipients demonstrated 7% to 29% donor type A hemoglobin in peripheral blood during the 6 months of postnatal observation.

Table IV. G418-resistant colonies

	Sheep (%) (n = 4)		*Human (%) (n = 3)*	
	Adult	*Fetal*	*Adult*	*Fetal*
Colony-forming units, mixed	12	25	0	5.4
Colony-forming units, granulocyte-macrophage	7	20	2	6.0
Burst-forming units, erythroid	15	25	1	4.0
Colony-forming units, erythrocyte	15	15	1	12.6

made in utero. However, the sheep model may not be applicable to the human, so these findings, although encouraging, must be reproduced in a nonhuman primate model.

In utero hematopoietic stem cell transplantation in primates

Although the sheep model has provided many insights into fetal hematopoietic stem cell transplantation, application of these findings in humans is severely limited because sheep gestational physiology and immunology are so dissimilar from those of humans. Before this promising technique could be applied clinically, we felt that feasibility, safety, and efficacy would have to be demonstrated in the nonhuman primate, whose developmental biology and immunologic development are most similar to the human. We have subsequently established the feasibility of an in utero hematopoietic stem cell transplantation in fetal rhesus monkeys at 60 days of gestation.[24, 25]

The research plan is outlined in Fig. 4. Time-dated, pregnant rhesus monkeys *(Macaca mulatta)* were obtained from the California Primate Research Center at the University of California, Davis. Fetectomy was performed at 59 to 68 days of gestation and the liver was harvested as a source of hematopoietic stem cells. Donor cells were prepared by the same technique used in sheep hematopoietic stem cell transplant recipients. Aliquots at a final concentration of 1×10^7 nucleated cells were separated and frozen in 10% dimethyl sulfoxide and fetal calf serum until used. Each recipient fetus received 10^8 to 10^9 cells per kilogram of estimated fetal body weight. The sex of donor fetuses was determined by anatomic dissection at the time of liver harvest. The sex of all but three of the recipient fetuses was determined before transplantation by chorionic villus sampling and direct-preparation karyotype analysis (Fig. 5) or by ultrasonography (Fig. 6), and these received cells from donors of the opposite sex. The sex of three other recipient fetuses was not known at the time of donor cell infusion; these fetuses received an equal mixture of cells from male and female donors. Transplantation of donor cells (10^8 to 10^9 cells per kilogram of fetal weight in 1 ml volume) was performed by injection into the recipient fetus through a 25-gauge spinal needle placed into the fetal peritoneal cavity under ultrasonographic guidance (Figs. 7 and 8).

In utero hematopoietic stem cell transplantation was performed in five fetal rhesus monkeys at 60 days' gestation. Five of the six monkeys with transplants survived to term. The first transplant attempted at the 60 days' gestation time point (No. 17908) was observed to have abruptio placentae during the injection and spontaneously aborted. Three of the five hematopoietic stem cell transplant recipients surviving to term are alive and well 3 to 20 months of age without evidence of graft-versus-host disease by physical examination, liver function tests, and lip biopsy. One rhesus monkey died

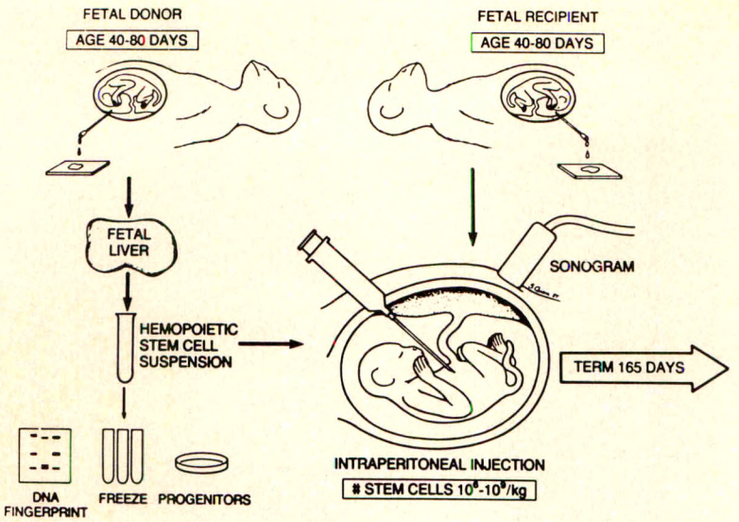


Fig. 4. In rhesus monkey model for in utero hematopoietic stem cell transplantation, there was no naturally occurring polymorphism to allow postnatal identification of donor cells. For that reason only cross-sex transplants were performed, that is, male donor in female recipient and vice versa. Sex of fetal donor was determined by anatomic dissection at the time of fetectomy. Sex of recipient fetus was determined by ultrasonography (if later than 70 days' gestation) or direct-preparation karyotype analysis of a chorionic villus (if earlier than 70 days' gestation). Fetal hematopoietic stem cells were cryopreserved until transplantation was performed by intraperitoneal injection under ultrasonographic guidance.

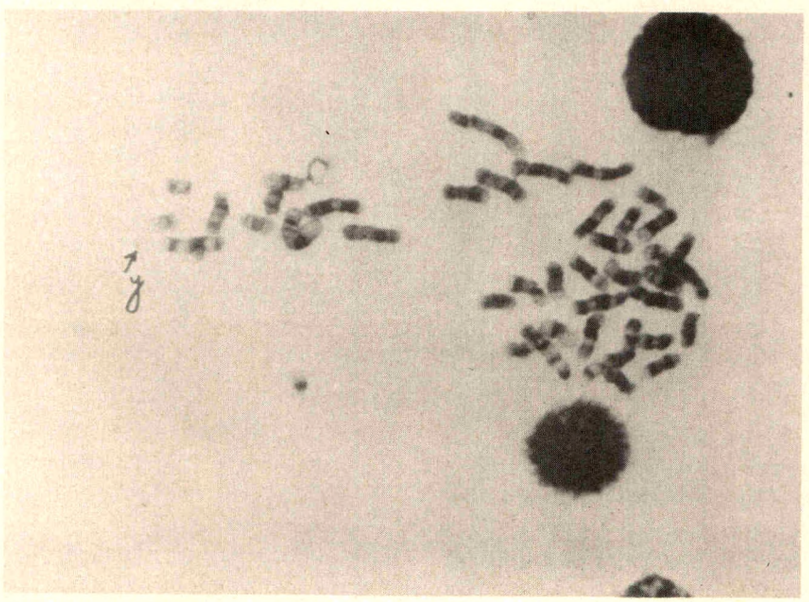


Fig. 5. Karyotype analysis of chorionic villi prenatally, and peripheral blood leukocytes and bone marrow cells allowed the identification of donor cells of opposite sex to the recipient.

shortly after birth of sepsis from chorioamnionitis despite emergency cesarean section. A second rhesus monkey died at 2 weeks of age of severe enteritis, which is endemic in the primate colony.

Hematopoietic stem cell transplants were successful as evidenced by significant levels of donor hematopoietic cell lines detected in recipients.

The pluripotential nature of the fetal hematopoietic stem cell is demonstrated by hematopoietic chimerism found in lymphoid, erythroid, and myeloid cell lines.

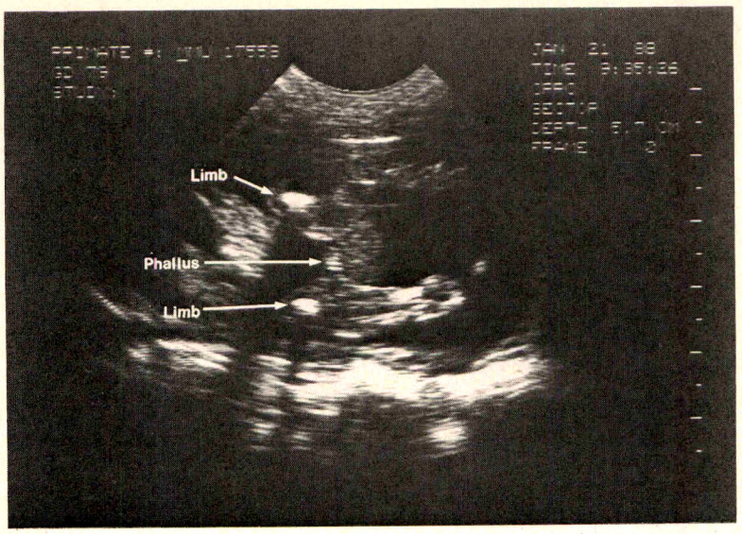


Fig. 6. Ultrasonographic image of fetus with visible phallus confirming male sex.

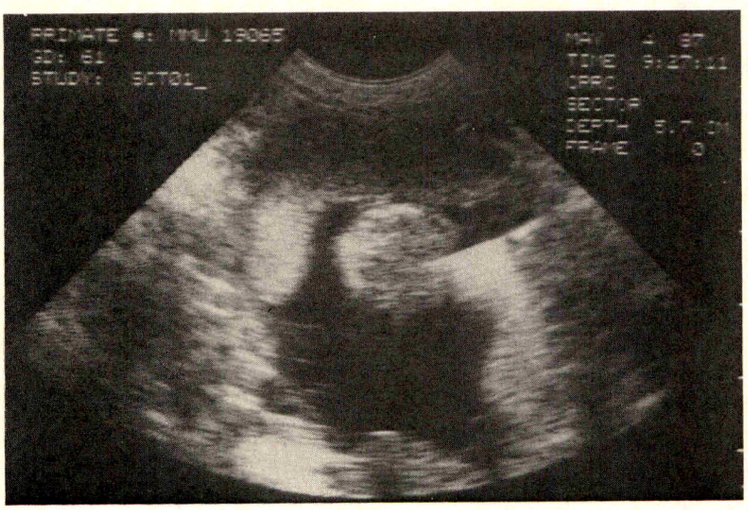


Fig. 7. Ultrasonographic image of hematopoietic stem cell transplantation demonstrating placental disk superiorly and the fetal abdomen in cross section with needle entering peritoneal cavity from right.

In all cases the presence of donor hematopoietic progenitors in the recipient bone marrow was accompanied by the presence of donor cells in the recipients' peripheral blood (Table V), suggesting functional expression of the engrafted donor stem cells. The engraftment in these hematopoietic chimeras has been stable for long periods of time without loss of progenitors from bone marrow or functional expression in peripheral blood (Fig. 9).

Surveillance tests for clinical and subclinical graft-versus-host disease including liver function tests and lip biopsies have been normal. There appears to be complete tolerance of the host by the graft and vice versa. Extensive postmortem histologic examination of the recipients that died in the newborn period (Nos. 19451 and 23857) revealed no evidence of graft-versus-host disease.

The ability of a preimmune fetal recipient to maintain sustained engraftment and expression of donor hematopoietic stem cells throughout gestation and after birth is dependent on its becoming immunologically tolerant of the allogeneic hematopoietic stem cells.[26, 27] Fetal tolerance resulting in permanent chimerism has been shown to occur in nature in twins with shared placental circulation[26, 27] and has been experimentally induced in animal models.[28, 29] Little is known about when in gestation and by what mechanism tolerance is lost. Our results in rhesus monkeys suggest that tolerance

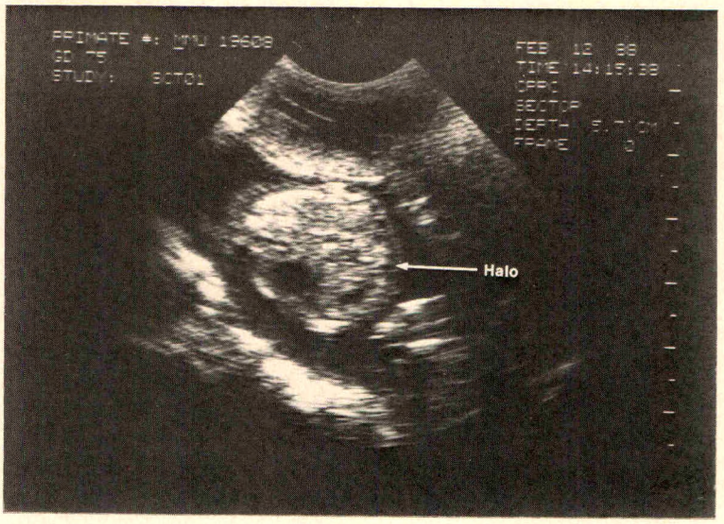


Fig. 8. Postinjection ultrasonographic image demonstrating peritoneal "halo" effect confirming intraperitoneal location of hematopoietic stem cell suspension.

persists until at least 68 days' gestation (~16½ weeks in human gestation). The most important advantage of using fetal hematopoietic stem cells as donor material is that graft-versus-host disease may be ameliorated or eliminated. None of our chimeric sheep[30] or our chimeric monkeys have evidence of graft-versus-host disease. If the preimmune fetal recipient is tolerant of a graft, it follows that donor preimmune hematopoietic stem cells from fetuses of the same gestational age or younger should be tolerant of the recipient. The use of preimmune fetal hematopoietic stem cells for in utero transplantation may obviate three of the most serious problems with postnatal bone marrow transplantation: the need for ablation of the native bone marrow, the possibility of graft rejection, and the threat of graft-versus-host disease.

In utero transplantation of fetal hematopoietic stem cells in rhesus monkeys has proved technically feasible without need for ablation of native marrow or for long-term immunosuppression. Engraftment without rejection or graft-versus-host disease suggests that tolerance to foreign antigen can be induced by at least 68 days' gestation in the rhesus monkey (approximately 16½ weeks in human gestation). The techniques used for in utero hematopoietic stem cell transplantation in rhesus monkeys can be readily applied in humans and may offer the first effective therapy of genetic disorders in utero.

Transplantation of fetal islet cells

Diabetes mellitus is a common disease that is a major source of morbidity and early death. Long-term management with insulin is possible, but perfect control of blood glucose (which appears to be necessary for the prevention of angiopathy) can be provided only by functioning pancreatic islet cells. For this reason there have been many attempts over the past 25 years to develop islet cell transplantation as a therapeutic modality.

Pancreatic islet cells have been used both as whole-organ and as cellular transplants, and both postnatal and fetal donor tissue has been used. Fetal tissue was initially thought to have several advantages: It was thought to be less immunogenic; the fetal pancreas has a higher proportion of islet cells to exocrine cells, and

Table V. Karyotype analysis of blood and bone marrow cells from rhesus monkey recipients of in utero hematopoietic stem cell transplantation

Animal no.	*Donor cells* (%)*		
	Lymphoid	*Erythroid*	*Myeloid*
19451	8.0†	—	—
23670	4.8†	12.5†	15.4†
23856	6.2	7.3	9.3
24479	2.9	5.3	8.5
24178	1.8	—	—
24175	2.4	—	—
24270	7.5	—	—

*Karyotype analyses performed at 1 month of age. Chromosomal analysis of lymphoid cells was performed on peripheral blood cells. Bone marrow cells cultured in the presence of erythropoietin represented the erythroid cell line. Percentage of donor myeloid cells was determined in cultures of bone marrow cells with monkey phytohemagglutinin-leukocyte–conditioned media.

†These rhesus monkeys received an equal mixture of male and female donor cells. The percentage of donor cells given was derived on the basis of the assumption that donor cells of both sexes engrafted similarly; the actual observed amount is 50% of the indicated value.

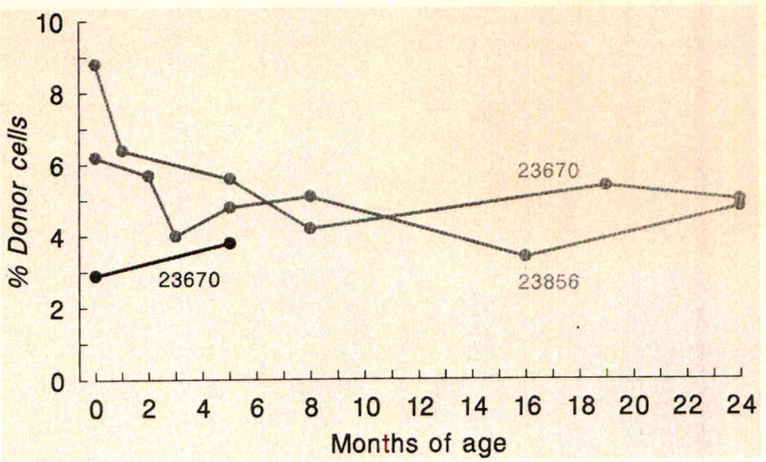


Fig. 9. Engraftment of peripheral blood leukocytes has persisted during 24 months of observation of three in utero hematopoietic stem cell transplant recipients.

in many countries fetal tissue is more readily available than adult cadaveric pancreas.[31]

Early studies in rodents of fetal pancreas grafts showed that fetal islet cells grew and functioned when transplanted to syngeneic animals and were capable, even in small quantities, of reversing alloxan-induced diabetes.[32, 33] In addition, they suggested that fetal islet allografts had a better survival than adult islets. However, more recent work[34] has confirmed that allografts of fetal pancreas are rejected within several weeks and that this rejection occurs despite treatment with cyclosporine. It appears, in fact, that late-gestation fetal islet tissue contains a large quantity of highly immunogenic lymphoid tissue.[35] It is possible that islet cells from earlier gestation would be less immunogenic, but it is unclear whether functional capacity would be adequate at very early gestational ages. It is known, for example, that early human fetal islet cells in culture do not respond to a glucose challenge before 25 weeks' gestation.[36] However, it is also clear that transplanted early fetal islet cells will grow, develop, and eventually function normally in a syngeneic or athymic host. One recent study documented normal glucose tolerance curves in alloxan-treated nude mice 6 to 8 weeks after transplantation of human fetal islet cells of 13 to 17 weeks' gestation.[37] Attempts have been made to decrease the immunogenicity of fetal islet cell grafts, by means of a number of different techniques. It was noted in mice that immunogenicity could be decreased by culturing the islet cells in media with a high concentration of oxygen.[38] Although results have been less impressive in the rat model,[39] this concept has undergone further investigation and the technique has been used in a number of clinical trials.[40] Others have attempted to decrease immunogenicity by decreasing the number of passenger lymphocytes in the graft. This can be done by exposure to ultraviolet radiation,[41] treatment with specific antibodies,[42] and passaging the islet cells through an interim host, usually the nude mouse.[43] The efficacy of these techniques in large animal and human systems has yet to be proved.

One further technique of interest is encapsulation of islet cells with a semipermeable membrane,[44] which allows glucose and insulin to pass but inhibits larger molecules such as immunoglobulins. This concept has great appeal, and initial results in animals have been encouraging.

Transplantation of fetal dopaminergic neurons

Parkinson's disease is a condition in which there is a failure of dopaminergic input from the substantia nigra to the striatum, resulting in progressive tremor, extrapyramidal signs, and hypokinesia. The disease is probably caused by degeneration of the dopamine-producing cells in the substantia nigra. The concept of cellular transplantation of dopamine-producing cells, with stereotaxic surgery, has opened an exciting new vista for the treatment of this disease and has stimulated a tremendous amount of experimental research over the past decade.

There were several problems with the idea from the outset. It is well known that adult neurons do not divide, and it appeared doubtful that these cells, even if they were to survive and proliferate, would establish the appropriate connections in the brain to function in the desired manner. The second problem was that of rejection. Despite the fact that the brain is relatively "privileged" immunologically,[45] it can clearly mount an immune response to foreign tissue.[46] The solution to both of these problems lay in the use of fetal tissue from very early gestation, which is less immunogenic and still has the ability to divide and establish neuronal connections.[47]

Initial experiments in rodents proved that cells taken from fetal brain, adrenal gland, or spinal cord would survive in an autologous recipient, would grow and

divide, and would function, even so far as to reverse symptoms in various models of parkinsonism.[48] These results have been duplicated in allogeneic transplants in nonhuman primates.[49] Even xenografts (mouse to rat, monkey to rat, and human to rat) have been shown to function and survive if the recipient is immunosuppressed with cyclosporine.[50] There is some evidence that rejection can be decreased and function improved by in vitro culturing of the cells before transplantation.[51]

Further studies with models of Parkinson's disease in nonhuman primates have confirmed the efficacy of transplanting fetal dopaminergic neuronal tissue. This has been done successfully by a number of investigators without immunosuppression and with relatively long-term effect.[52,53] It is interesting that preliminary human trials with autologous adrenal cells used as the donor tissue have been largely unsuccessful.[54] This is probably due to the inability of adult tissue to grow, divide, and function in a transplant situation. Clinical trials with human fetal tissue are under way,[55] and the results of these trials are awaited with great interest.

Transplantation of fetal liver cells

Transplantation of hepatocytes is an appealing alternative to orthotopic liver transplantation in patients with a hepatic enzyme deficiency such as ornithine transcarbamylase deficiency or a factor deficiency such as hemophilia, since they have otherwise normal liver. An additional advantage, unlike the procedure for whole organ transplantation, is that hepatocytes can be "banked" by cryopreservation until an appropriate donor is found. Finally, hepatocellular transplantation is minimally invasive and would not preclude orthotopic liver transplantation if it failed.

Fetal hepatocytes, although functionally immature, have been shown to be less immunogeneic than postnatal hepatocytes, evolving little or no immune response from the host.[55] The use of fetal hepatocytes in transplantation might have the advantage of reduced immunogenicity and a decreased need for immunosuppression. Thus the fetus may be not only the ideal donor of hepatocytes but also the ideal recipient.

Advances in prenatal diagnosis now make it possible to identify fetuses with heritable metabolic disorders and factor deficiencies as early in gestation as 8 to 10 weeks,[18] well before the onset of fetal immunocompetence. In utero hepatocellular transplantation could reconstitute the deficiency before any clinical manifestation of the disease and result in long-lasting tolerance of the host, without need for long-term immunosuppression. In utero hepatocellular transplantation is a potential therapy in diseases currently incurable except by orthotopic liver transplantation. Numerous questions that need to be addressed experimentally include: (1) What is the best technical approach, i.e., intraperitoneal infusion, umbilical vein infusion, or intraparenchymal injection? (2) What is the role of hepatotropic factors in engraftment of fetal hepatocytes? (3) Could induction of tolerance in the recipient be induced by pure hepatocellular transplantation or are hematopoietic stem cells required? Investigation of in utero hepatocellular transplantation in animals with long gestations will be essential to the assessment of feasibility and potential clinical applicability for treatment of hepatic enzyme deficiencies in man.

Ethical issues in the use of fetal tissue for transplantation

Tissue obtained from a deceased human fetus is entitled to the same dignity and respect accorded tissue obtained from a deceased adult. In itself, the use of fetal tissue is not ethically objectionable, but because it becomes available as a result of a mother's decision to end her pregnancy, a potential ethical dilemma is created. The possibility is raised that the use of fetal tissue might encourage abortion (or pregnancies) that would not have occurred otherwise.

Although legalized abortion remains a hotly debated, controversial issue, a consensus has been forming regarding the ethical use of fetal tissue in research and clinical transplantation. A National Institutes of Health panel recently released their recommendations affirming that use of fetal tissue is ethically unobjectionable as long as certain guidelines are followed.[56] The Committee on Ethics at Stanford University Medical Center similarly affirmed the ethical use of fetal tissue.[57]

To avoid potential ethical problems in the use of fetal tissue, mothers should not be allowed to benefit in any way from the use of fetal tissue they have elected to abort. This should include any compensation of expenses associated with the abortion. Similarly, the physicians performing abortions should not be allowed to benefit from the subsequent use of fetal tissue. Physicians may consciously or unconsciously influence a patient's decision to have an abortion if they have a direct interest in the use of fetal tissue. Some have suggested that to avoid any conflict of interest even academic incentives (e.g., coauthorship of publications or grant support) should be excluded from physicians performing abortions.[58] The mother should not be allowed to designate a recipient of fetal tissue as this also might influence a woman's decision to have an abortion. These guidelines help to ensure that a woman's decision to terminate a pregnancy is in no way influenced by the subsequent use of fetal tissue. Fetal tissue obtained under these ethical guidelines can be used for transplantation research or therapeutic interventions.

Comment

The fetus should be the ideal donor of hematopoietic stem cells for transplantation. During human gestation hematopoietic stem cells are first found in the yolk sac at about the fourth week of gestation. It is from

here that the liver (sixth week of gestation) and spleen (seventh week of gestation) are seeded. From about the fourteenth to the twentieth week of gestation, hematopoiesis finally switches to the bone marrow.[59] The bone marrow is seeded by the liver as the liver was seeded by the yolk sac. Therefore fetal liver should be an ideal source of hematopoietic stem cells that are primed to seed waiting bone marrow.[60] Like postnatal bone marrow hematopoietic stem cells, fetal liver hematopoietic stem cells are pluripotential and have the capacity to differentiate into a complete repertoire of erythroid, myeloid, thromboid, and lymphoid cell lines.[61, 62] Although hematopoietic stem cells from fetal liver may not be as efficient as postnatal bone marrow hematopoietic stem cells in terms of the number of colony-forming units seen when the same number of nucleated cells is injected,[63] fetal liver cells have the advantage of being primed and ready for hematogenous seeding of the recipient marrow.[59]

The most important advantage of fetal hematopoietic stem cells as donor material is that graft-versus-host disease may be ameliorated or avoided. If the fetal host is immunotolerant of a graft (see below), it follows that donor fetal hematopoietic stem cells should be immunotolerant of the recipient, because the lymphocytes that respond to specific foreign antigens are still "naive." Lethally irradiated mice reconstituted with allogeneic fetal stem cells do not develop graft-versus-host disease.[64-67] In humans, lymphocytes capable of causing graft-versus-host disease are detectable in the thymus by the fourteenth week of gestation but are not detected in the liver until the eighteenth to twentieth week of gestation.[60] These observations led clinicians doing postnatal bone marrow transplants to use fetal liver hematopoietic stem cells from donors who were between 8 and 14 weeks' gestation. Indeed, no instances of fatal graft-versus-host disease have occurred in human recipients of fetal liver hematopoietic stem cells >14 weeks' gestation.[68-70]

The fetus may be an ideal host for transplantation of hematopoietic stem cells, not only because of its ontologic readiness for engraftment before 20 weeks' gestation but also because the fetus in very early gestation is immunotolerant and permits foreign grafts without rejection. Sustained engraftment of allogenic cells in a recipient fetus is critically dependent on fetal immunologic tolerance of the graft. Fetal tolerance resulting in permanent chimerism has been documented to occur naturally in animal and human twins with shared placental circulation,[26, 27, 71, 72] and fetal tolerance has been demonstrated experimentally in several animal models.[28, 29, 73] Although transplantation immunity develops early in all mammals of long gestation, there is a period in early gestation when the fetus is uniquely tolerant of foreign antigen, but exactly when in gestation tolerance is lost is not known.[74] In the sheep fetus, prolonged survival of allogeneic skin grafts before 67 days' gestation has been demonstrated, while grafts placed after 77 days' gestation are rejected vigorously.[75-77] In a previous study we observed chimerism in three of five lambs transplanted at 85 to 105 days' gestation with allogeneic adult bone marrow cells, suggesting that even after the onset of immunocompetence the fetus is uniquely susceptible to tolerance induction.[78] These animals sustained chimerism but ultimately died of graft-versus-host disease. We then showed that using fetal liver hematopoietic stem cells rather than adult bone marrow cells and transplanting them earlier in gestation yielded sustained engraftment without evidence of either rejection or graft-versus-host disease.[30] This study provides strong support for the concept that the fetus is an ideal recipient, as well as an ideal donor for hematopoietic stem cell transplantation.

The concept of in utero transplantation of hematopoietic stem cells is so appealing that it has already been attempted clinically. Linch and Rodeck[79] reported transplanting maternal bone marrow hematopoietic stem cells (depleted of T cells, thought to cause graft-versus-host disease, by monoclonal antibody treatment) into an 18-week fetus with severe hemolytic anemia. The fetus survived after multiple standard intrauterine transfusions, but there was no evidence of engraftment after birth. This is not surprising for two reasons. Adult (maternal) bone marrow is not ideal as donor material, especially since attempts to reduce graft-versus-host disease by depleting T cells[80, 81] may decrease the chance of successful engraftment by removing T-cell hematopoietic factors. The attempted transplant may have been too late (18 weeks) to allow engraftment without rejection.[82, 83] Certainly other attempts will be made, but clinical application of this promising therapy desperately needs evaluation in an appropriately rigorous animal model.

From a practical standoint, fetus-to-fetus hematopoietic stem cells transplants may allow the use of allogeneic stem cell grafts from unmatched donors and possibly even pooled donor cells, thus obviating the need for human leukocyte antigen typing and search for a specific donor as is generally required for postnatal bone marrow transplantation. It also would remove the need for pretreatment with radiation and/or chemotherapy, as is necessary with immunocompetent recipients who have the capacity to reject an allogeneic graft.[3, 5, 15] Another reason for pretreatment of the postnatal recipient is to rid the bone marrow stroma of its occupant cells to make room for the implantation of injected hematopoietic stem cells.[3] In the period before the twentieth week, not only does the human fetus have a depleted stromal environment, but it is programmed for the reception of hematopoietic stem cells.[59] Fetal immunotolerance and stromal preparedness obviate the need for host preparation with chemotherapy

and/or irradiation, a significant disadvantage of postnatal bone marrow transplantation.

In summary, cellular transplantation may provide a means for replacing absent or defective cells without the need for a solid organ transplant. The fetus may be an ideal donor for these cells in several situations, because of its unique immunologic and functional attributes. By the same token, the fetus makes an ideal recipient for hematopoietic stem cells and, potentially, for many other cellular transplants. Experimental work with fetal hematopoietic stem cells, islet cells, and dopaminergic cells suggests that the fetus may soon play an important role in the field of cellular transplantation.

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LETTERS TO THE EDITORS

Methotrexate treatment of tubal pregnancy

To the Editors: Stovall et al. (Stovall TG, Ling FW, Buster JE. Reproductive performance after methotrexate treatment of ectopic pregnancy. AM J OBSTET GYNECOL 1990;162:1620-4) made a worthwhile contribution to our knowledge of methotrexate treatment of tubal pregnancies.

The authors stated that the intrauterine pregnancy rate of 90.1% in their series is superior to the 49% to 70% rate for linear salpingostomy in a review by Vermesh.[1] These two groups may not be comparable, and the pregnancy rates seem to have different derivations. The salpingostomy group included patients with apparently more severe tubal damage (e.g., dilatation ≤6 cm for inclusion; ruptured fallopian tubes). The 90.1% pregnancy rate derived by Stovall et al. is intrauterine pregnancies per total conceptions. The pregnancy rates in the salpingostomy review are intrauterine pregnancies per total patients.

The authors note that the principal value of this approach is to bypass the risk, cost, and morbidity of surgical intervention. Because laparoscopy was performed on all subjects to establish the diagnosis, one might take the point of view that the principal morbidity of endoscopic salpingostomy was already incurred and considering the cost of methotrexate and related toxicity monitoring, endoscopic salpingostomy would be the least costly and least morbid approach.

Bruce G. Bateman, MD

Department of Obstetrics and Gynecology, Box 387, University of Virginia Health Sciences Center, Charlottesville, VA 22908

REFERENCE

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Reply

To the Editors: We thank Dr. Bateman for his letter with regard to our article. He is correct in stating that the surgical series of Vermesh et al. and our series of medically treated patients may not be comparable in terms of future pregnancy outcome. Because of an aggressive screening program at our institution, patients with ectopic pregnancy are diagnosed earlier than those in the past. Thus the ectopic pregnancies are smaller and associated with less tubal distortion than are patients included in most surgical series or patients in our institution who require surgical therapy.

The favorable cost/benefit ratio of methotrexate to laparoscopic surgical therapy is arguable when laparoscopy is used to diagnose the ectopic pregnancy. To avoid laparoscopy, we developed and implemented a nonsurgical algorithm for the diagnosis of ectopic pregnancy and are able to diagnose as well as treat ectopic pregnancy without surgery.[1] On the other hand, one might surmise that even when laparoscopy is needed for diagnostic purposes, less tubal damage is ultimately done with medical therapy than if the tube is cut, cauterized, or lased. In addition, it should be remembered that if conservative surgery is undertaken at the time of laparoscopy, human chorionic gonadotropin titer monitoring is required after surgery to detect the 5% of patients who have persistent trophoblastic activity. The cost and effort of this follow-up must be factored in whether or not the patient requires additional surgery or methotrexate therapy.

In our article, the pregnancy rate is reported in two manners. First, 78.6% of patients attempting pregnancy were able to conceive, whereas 90.1% of patients who conceived had intrauterine pregnancies. The 78.6% is still superior to the 49% to 70% rate for linear salpingostomy reported in the review by Vermesh et al. Finally, we accrued over 35 pregnancies after methotrexate therapy. The intrauterine pregnancy rate is 88% with a recurrent ectopic rate of 12%.

Thomas G. Stovall, MD, Frank W. Ling, MD, and John E. Buster, MD

Department of Obstetrics and Gynecology, College of Medicine, University of Tennessee, 853 Jefferson Ave., Memphis, TN 38103

REFERENCE

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Nonimmune hydrops in fetal Niemann-Pick disease

To the Editors: Meizner et al. recently ascribed a new cause to nonimmune hydrops fetalis—Niemann-Pick disease (Meizner I, Levy A, Carmi R, et al. Niemann-Pick disease associated with nonimmune hydrops fetalis. AM J OBSTET GYNECOL 1990;163:128-9). Niemann-Pick disease, both type A[1] and type C,[2] have previously been implicated in nonimmune hydrops fetalis, as have Wolman's disease,[3] Salla's disease,[4] and β-glucuronidase deficiency,[5,6] extending the list of lysosomal storage diseases that may cause hydrops.

Jaclyn Vidgoff, PhD

Oregon Tissue Bank, Emanuel Hospital and Health Center, 2801 N. Gantenbein Ave., Portland, OR 97227

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Reply

To the Editors: We appreciate the interest of Dr. Vidgoff in our work. With reference to her letter we would like to point out the following:

Dr. Vidgoff states that Niemann-Pick disease, both type A and type C, have previously been implicated in nonimmune hydrops fetalis and gives two references to support her statement. Is that really so?

In the first article by Schoenfeld et al.[1] neither the term nor the entity nonimmune hydrops fetalis is mentioned. The article deals with placental findings associated with Niemann-Pick disease and does not indicate any of the features of hydrops fetalis. Prenatal ultrasonographic findings of nonimmune hydrops fetalis are not the subject of the article, which describes the ultrasonographic (placental only), biochemical, and histochemical aspects of four affected fetuses with Niemann-Pick disease.

The second article mentioned by Dr. Vidgoff is by Maconochie et al.,[2] who reported on isolated fetal ascites in two fetuses affected by Niemann-Pick disease. Again, neither the term nor the entity of nonimmune hydrops fetalis is mentioned in this case report. Fetal ascites was the only ultrasonographic and clinical finding reported. Does this sole finding indicate "a previous description" of nonimmune hydrops fetalis as suggested by Dr. Vidgoff? Where are other findings associated with nonimmune hydrops fetalis, such as hydrothorax and generalized edema?

The sonographer searches for the classic findings of skin thickening >5 mm, placental enlargement >5 cm, ascites, pleural effusion, pericardial effusion, and polyhydramnios. In some studies, ascites alone is considered diagnostic of nonimmune hydrops fetalis.[3] Other studies are purposely skewed to severe nonimmune hydrops fetalis, requiring serous effusions in two body cavities or a serous effusion in one body cavity in addition to anasarca for the diagnosis.[4] This lack of precision leads to variation in the cases included in series of nonimmune hydrops fetalis.

Although it is true that ascites can be seen in the hydropic state, when it occurs alone it is often from a local rather than a generalized condition. Ascites has often been noted to occur with urinary and intestinal tract obstruction.[5] Fetuses that display only ascites, unassociated with generalized hydrops, generally have a better prognosis and should be considered separately. We share what most authors believe, that the diagnosis of nonimmune hydrops fetalis should be reserved to more than one serous cavity effusion (ascites, pleural effusion, or pericardial effusion) or a serous effusion in conjunction with anasarca.[6,7]

Because nonimmune hydrops fetalis was not present in any of the articles suggested by Dr. Vidgoff and because ascites alone is not sufficient for diagnosis of nonimmune hydrops fetalis, our case in which the fetus had both ascites and pleural effusion is the first to show true nonimmune hydrops fetalis in association with Niemann-Pick disease.

Israel Meizner, MD
Ultrasound Unit, Division of Obstetrics and Gynecology, Soroka Medical Center, Beer-Sheva, Israel 84101

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Low-risk cesarean section prophylaxis issue still unsettled

To the Editors: We read with considerable interest the article by Ehrenkranz et al. (Ehrenkranz NJ, Blackwelder WC, Pfaff SJ. Infections complicating low-risk cesarean section in community hospitals: Efficacy of antimicrobial prophylaxis. Am J Obstet Gynecol 162:337-43). We agree that the study objectives are important, but believe that the study design fails to adequately address those objectives. The selection criteria for low-risk patients included both nonlaboring patients with intact membranes and laboring patients with membranes ruptured up to 12 hours. These selection criteria have effectively included both true low-risk patients and high-risk patients (as defined by Gilstrap and Cunningham[1]).

The efficacy of antibiotic prophylaxis is used by attending physicians (a listed objective) cannot be evaluated for clinical use when the antibiotic used is not stated. Which antibiotic(s) was used, how many doses were given, and whether one antibiotic was more (or less) effective than the others are critically important when evaluating overall prophylaxis efficacy. The conclusions stated by the authors assume that all antibiotic prophylaxis is equivalent in this setting, and the use of any antibiotic in any regimen is effective in low-risk cesarean section settings.

The authors state that "Antibiotics administered up to 24 hours before operation were regarded as having been given for the purpose of antimicrobial prophylaxis." We are unaware of any data that antibiotics administered 24 hours before surgery have any effect on postoperative infectious morbidity. Although the authors later define the term *timely prophylaxis*, what was the purpose of their original definition? Were those patients given timely prophylaxis in addition? If so,

were these patients considered with patients who did not receive the other extraneous doses of antibiotics in evaluation of infectious morbidity?

The authors stated that new manifestation of pyuria was a criterion for urinary tract infection, and that fever and purulent sputum was a criterion for lower respiratory infection. We believe that neither of these criteria is sufficient for their stated diagnosis.

Finally, the statement was made that the administration of antibiotics failed to result in serious diarrhea; however, they failed to mention two other important potential adverse effects of antibiotic administration—anaphylaxis and bacteriologic shifts in colonizing flora and resistance patterns. Gibbs et al.[2] have shown a significant decrease in low-virulence organisms with a concomitant increase in gram-negative facultative anaerobic organisms after antibiotic prophylaxis.[2] This must be considered when making the recommendation to administer antibiotics to more than 100,000 women annually. Although anaphylaxis is rare with β-lactams (about 1 in 10,000), the administration of antibiotics to an additional 165,000 women will likely result in some cases of anaphylaxis. Death has resulted from anaphylaxis as a result of prophylactic administration of antibiotics.[3]

In conclusion, we believe that study design in this paper cannot clearly identify an advantage of prophylactic administration of antibiotics to true low-risk patients undergoing cesarean section, and believe that the existing data suggest that the risks outweigh the benefits for this indication.

Mark Pearlman, MD, Sebastian Faro, MD, PhD, Hunter Hammill, MD, and Maurizio Maccato, MD

Baylor College of Medicine, Department of Obstetrics and Gynecology, Section of Infectious Disease, Houston, TX 77030

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Reply

To the Editors: Pearlman et al. raised important points about our article.

Inherent infection risks are defined in untreated populations. Gilstrap and Cunningham[1] found 94.6% of untreated indigent women with membrane rupture >6 hours had infection after cesarean section. In our low-risk, untreated private patients, endometritis and wound infection totalled only 4.1%. Review of our case-control groups reveals 50% of both had no membrane rupture, and the proportions with rupture ≤6 and >6 hours were similar. In nonindigent women, membrane rupture durations ≤15 hours have not been associated with infection.[2-4]

Since 1970 symptomatic urinary tract infection criteria have included pyuria ≥10 white blood cell count.[5, 6] Probable lower respiratory tract infection is authoritatively defined as new fever, purulent sputum, and leukocytosis.[7] Inasmuch as concurrent leukocyte counts were frequently unavailable, we used the first two criteria. Use of additional criteria could not have increased our low infection rates.

In our cohort study, to seek any measureable prophylactic efficacy, we evaluated antibiotics administered up to 24 hours preoperatively. In the case-control study we collected extra information to explore whether timely prophylaxis (from 2 hours before to 4 hours after incision) might be efficacious.

We are mystified that Pearlman et al. "are unaware of any data that antibiotics administered 24 hours before surgery have any effect on postoperative infectious morbidity." Oral antibiotics administered only the day before bowel surgery are well known to prevent postoperative infections. These antibiotics numerically diminish enteric mucosal-associated bacteria below levels permitting operative site infection.[8] Systemically absorbed antimicrobial agents administered up to 24 hours before cesarean section might similarly diminish genital mucosal-associated bacteria—a potential cause of intrauterine contamination.[1]

Of 906 women (49%) in our cohort who received antibiotics prophylactically, over 60% received a first- or second-generation cephalosporin and 90% received one of these cephalosporins or a penicillin. We made no conclusion from our data about relative efficacies of different regimens.

Currently 50% of low-risk patients receive three or more antimicrobial doses. Martens, et al.[9] found one cephalosporin dose less likely to induce colonization by resistant bacteria than three. If 100% of patients receive one dose and both regimens are equally effective, a net reduction in antibiotic-resistant bacteria is entirely possible. We suggested three cefazolin doses on the basis of well-designed studies, but indicated that one-dose courses had potential annual savings of $9,400,000.

Finally, concern about anaphylaxis has been expressed. Up to 40% of hospitalized patients receive antimicrobial agents; however, documented cephalosporin-induced anaphylaxis is extremely rare. For previously unidentified anaphylaxis-susceptible women, an operating room is the safest place for such a rare occurrence, with life-support facilities immediately available. Cephalosporins administered only after cord-clamping pose no risk to infants.

In summary, we do not believe our Houston colleagues have offered a sufficiently strong rebuttal to the evidence from our study to alter the interpretations we have drawn.

N. Joel Ehrenkranz, MD, Sandra J. Pfaff, BSN, and Dolores Poppe, RRA

Florida Consortium for Infection Control/Quality Medical Care, 5901 Southwest 74th St., South Miami, FL 33143

William C. Blackwelder, PhD, Diane E. Yerg, MA, MSPH, and Richard A. Kaslow, MD

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Westwood Bldg., Room 739, Bethesda, MD 20892

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Chlamydial infection and pregnancy outcome

To the Editors: Ryan et al. presented data on *Chlamydia trachomatis* infection and pregnancy outcome (Ryan GM, Abdella TN, McNeeley SG, Baselski VS, Drummond DE. *Chlamydia trachomatis* infection in pregnancy and effect of treatment on outcome. AM J OBSTET GYNECOL 1990;162:34-9). Of 2433 women who were chlamydia-positive at their first prenatal visit, 1323 (54%) were judged to have been adequately treated on the basis of a negative endocervical chlamydial culture after erythromycin therapy. A total of 9111 women from a similar demographic background were chlamydia-negative at their first prenatal visit. Neither group were recultured for chlamydial infection during the last trimester. The pregnancy outcome in 1110 chlamydia-positive women was documented. Details about intercurrent antibiotic therapy were not presented. Sexual partners were neither screened nor given antichlamydial therapy and thus provided a route for reinfection in those women who were treated.

The risk of chlamydial infection to infants born of infected mothers ranges from 50% to 70%.[1] Of 1083 live births to chlamydia-positive mothers, 541 to 812 infants were at risk of acquiring the infection. Furthermore, neonatal conjunctivitis could have developed in 20% to 50% of infected infants, whereas 30% of those with nasopharyngeal infection could have had a chlamydial pneumonia. Asymptomatic prolonged vaginal, rectal, and pharyngeal carriage have been reported in up to 15% of infants infected with chlamydia.[2] This could have serious medical, social, and legal implications inasmuch as culture-proved chlamydial infection is used as a possible indicator of child sexual abuse. However, no information was given as to whether these children were being followed up or recalled for therapy.

Postpartum endometritis has been reported in association with *Chlamydia trachomatis*, developing 2 to 3 weeks after delivery.[3] This can occur in 30% to 60% of women with untreated antepartum chlamydial infection. Tubal damage may be a complication. However, cases were not documented in this study.

The most effective way to prevent neonatal chlamydial infection and puerperal morbidity is to treat all chlamydia-positive pregnant women. Their partners should receive appropriate therapy. Tests of cure should be performed and rescreening in the last trimester should be offered to those women previously found to be infected. There are no ethical justifications for further studies on pregnancy outcome in untreated chlamydia-positive women in whom risks of maternal morbidity and neonatal infection are well known.

G.E. Forster, MB, ChB, S. Estreich, MB, BS, and Y.S. Hooi, MB, ChB

Department of Genitourinary Medicine, The Ambrose King Centre, The Royal London Hospital, Whitechapel, London, England E1 1BB

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3. Hoyme UB, Kiviat N, Eschenbach DA. The microbiology and treatment of late postpartum endometritis. Obstet Gynecol 1986;68:226-32.

Reply

To the Editors: The letter from Forster, Estreich, and Hooi is in agreement with our basic contention that chlamydial infections during pregnancy do adversely impact the pregnancy outcome and that all chlamydia-positive pregnant women should be treated.

Another point raised in the letter was whether patients with a positive culture who are treated early in pregnancy should have another culture near term because reinfection can occur. We recently completed a study that shows that only 11.7% of women with previously treated *Chlamydia trachomatis* had positive cultures in labor. A more surprising finding was that 59% of those with positive chlamydia cultures in labor were associated with negative antenatal cultures at the time of their first prenatal visit. We concluded that in our population late pregnancy cultures would be useful in all patients, not just in those with previously positive cultures.

Subsequent treatment of children born to chlamydia-positive mothers was beyond the scope of the article as presented, but studies are under way in this regard.

Finally, we agree that on the basis of current knowledge it would not be justified to perform studies on untreated chlamydia-positive women; but at the time this study was begun between September 1, 1982, and January 1, 1984, several studies had indicated no relationship between a positive culture for chlamydia in pregnant women and premature rupture of the membranes, prematurity, or low birth weight. These studies were cited in our bibliography.

We thank the writers for their interest.

George M. Ryan, Jr., MD, MPH

Department of Obstetrics and Gynecology, College of Medicine, University of Tennessee, 853 Jefferson Ave., Memphis, TN 38103

Budd-Chiari syndrome, obstetric complications, and antiphospholipid antibodies

To the Editors: Budd-Chiari syndrome[1-3] has been associated with the presence of antiphospholipid antibodies (lupus anticoagulants or anticardiolipin antibodies), in addition to better-known obstetric complications. Diagnosis of antiphospholipid antibodies would be important in evaluation of a hypercoagulable state in the case reported by Ilan et al. (Ilan Y, Oren R, Shouval D. Postpartum Budd-Chiari syndrome with prolonged hypercoagulability state. AM J OBSTET GYNECOL 1990;162:1164-5) of a woman with both Budd-Chiari syndrome and preeclampsia.

The diagnosis would have added to treatment options when anticoagulation alone failed to halt progressive thrombosis by suggesting the addition of immunosuppressive agents or intravenous immunoglobulin infusions.[4] In addition to tests for antiphospholipid antibodies and the lack of evidence for protein C deficiency mentioned (presumably normal levels of protein C), other studies for hereditary and acquired thrombotic disorders would also be an important part of evaluation in such cases.[5] Perhaps the authors would share this information with us so we can learn from this woman's unfortunate outcome.

F. Susan Cowchock, MD
Jefferson Medical College, Division of Medical Genetics, Room 406, 1100 Walnut St., Philadelphia, PA 19107

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5. Salem HH, Mitchell CA, Firkin BG. Current views on the pathophysiology and investigations of thrombotic disorders. Am J Hematol 1987;25:463-74.

Reply

To the Editors: We appreciate the comments by Dr. Cowchock with regard to our case report of postpartum Budd-Chiari syndrome with prolonged hypercoagulability state. Budd-Chiari syndrome is known to occur in association with many hypercoagulable states including polycythemia vera; paroxysmal nocturia; hemoglobinuria; tumors such as adrenal carcinoma, hypernephroma, and hepatomas; radiation; oral contraceptives; proteins S, C, and antithrombin deficiencies; and inferior vena cava webs.[1-5]

During the past few years, cases of Budd-Chiari syndrome were described in patients with anticardiolipin syndrome.[4] The association of Budd-Chiari syndrome with pregnancy was first described by Chiari almost 100 years ago[5] and since then other case reports have been published.[6] These cases were all considered to be associated with the hypercoagulable state that occurs post partum.

The patient described in our article was unusual in that her hypercoagulable state lasted for months after delivery. We tried to find a basic systemic disorder that would explain the prolonged hypercoagulable state. Despite extensive workups including negative anticardiolipin test results, normal protein C and S levels, and searches for other malignancies, no explanation for the hypercoagulable state could be found.

We think the most likely reason for the prolonged and uneventful course was postpartum deep vein thrombosis that later initiated a cascade of prolonged hypercoagulable state with extension of pelvic vein thrombosis along the inferior vena cava.

Y. Ilan, MD, R. Oren, MD, and D. Shouval, MD
Department of Medicine A, Hadassah University Hospital, Jerusalem, Israel IL-91120

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2. Hoyumpa AM. Budd-Chiari syndrome in women taking oral contraceptives. Am J Med 1971;50:137-9.
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Was there really a difference with amnioinfusion?

To the Editors: Research data of Dr. Owens et al. (Owen J, Henson BV, Hauth JC. A propspective randomized study of saline solution amnioinfusion. AM J OBSTET GYNECOL 1990;162:1146-9) showing the apparent benefits of amnioinfusion is a different finding from our clinical experience.[1] The authors' rate of 19% postpartum endometritis in their control group is high. Perhaps it reflects the high rate of cesarean section in the controls.

Given that the treated and control groups had the same mean cord pH, I question the authors' impression about the apparent differences in fetal distress between the two groups.

In our hospital, amnioinfusion appears to be most effective for the fetal heart rate abnormalities that occur after artificial rupture of the membranes. I wonder whether the authors have also observed success in treating this iatrogenic fetal heart rate abnormality?

Robert C. Goodlin, MD
Department of Health and Hospitals, City and County of Denver, 777 Bannock St., Denver, CO 80204-4507

REFERENCE

1. Goodlin RC, Ingram M. Ultrasonic study of amnioinfusion. J Reprod Med 1990;35:439-40.

Reply

To the Editors: Dr. Goodlin's interest in our study is appreciated.

We performed a randomized controlled trial of amnioinfusion in 100 patients to determine its clinical utility in our population. Our article confirmed a trend toward a lower cesarean section rate for fetal distress as Nageotte[1] and Miyazaki[2] have previously reported.

As Dr. Goodlin correctly states, the incidence of cesarean delivery in our control population was higher than in the amnioinfusion group ($p = 0.2$). This may have partly accounted for the lower observed incidence of endometritis in the amnioinfusion patients. If the incidence of endometritis is stratified according to method of delivery, the following data emerge: of the 75 patients who underwent a vaginal delivery, 3 of 40 controls and none of 35 amnioinfusion patients had a postpartum infection. Similarly postcesarean endometritis developed in only one of 8 amnioinfusion patients compared with 8 of 17 control patients. Whereas neither of the stratified proportions are statistically significant at the $p = 0.05$ level, the trend, particularly in the postcesarean patients, is obvious. However, if amnioinfusion lowers the cesarean delivery rate, then it should have a favorable effect on the overall incidence of endometritis, which we demonstrated.

As stated in our conclusion, we would not expect a technique such as amnioinfusion to improve the cord pH with optimal labor management, the goal of which should be to effect delivery before fetal acidosis has occurred. Clearly the diagnosis of fetal distress is problematic and awaits more reliable and specific tests than a fetal monitor can offer.

Last, we have continued to use amnioinfusion at our institution, usually in the setting of persistant moderate to severe variable decelerations associated with labor in women with ruptured membranes. Although anecdotal, we continue to be impressed by the ability of this technique to correct these nonreassuring fetal heart rate patterns and permit progression to a vaginal delivery in many of these women.

John Owen, MD, and John C. Hauth, MD
Department of Obstetrics and Gynecology, University of Alabama at Birmingham, University Station, Birmingham, AL 35294

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1. Nageotte MP, Freeman RK, Garite TJ, Dorchester W. Prophylactic intrapartum amnioinfusion in ptients with preterm premature rupture of membranes. Am J Obstet Gynecol 1985;153:557-62.
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Coagulation changes on natural estrogens

To the Editors: I would like to comment on an article that was published in a supplement to the American Journal of Obstetrics and Gynecology (Notelovitz M. Estrogen replacement therapy: Indications, contraindications, and agent selection. Am J Obstet Gynecol 1989;161:1832-41).

In the section on coagulation and thrombosis, the authors of the study that describes the increase of platelet aggregation in patients who take conjugated equine estrogens were Leon Poller, Jean Thomson, and Jean Coope, not Poller, Thompson, and Thomas as printed in reference 92 in the Journal.[1] The same authors had previously published a study of clotting changes in these patients that included increases in factors VII and X and shortening of the prothrombin time after 3 months of estrogen therapy.[2]

The negative study by Aylward et al. was not published as a refereed article but appeared as a letter in the *British Medical Journal.* A number of studies have shown that estrogen therapy may accelerate blood clotting, depending on the dose and the potency of the preparation. This is not unexpected inasmuch as endogenous estrogens have a similar effect, both during pregnancy and in the menstrual cycle.[3]

Jean Coope, MD
Bollington Medical Centre, Bollington, Macclesfield, Cheshire, United Kingdom

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High CA 125 levels and spontaneous abortion

To the Editors: With great interest I read the article by Check et al. (Check JH, Nowroozi K, Vaze M, Wapner R, Seefried L. Very high CA 125 levels during early first trimester in three cases of spontaneous abortion with chromosomal abnormalities. Am J Obstet Gynecol 1990;162:674-5) describing high CA 125 serum concentrations in three cases of spontaneous abortion. The authors hypothesized that high CA 125 levels would be predictive for an increased risk of chromosomal abnormalities and spontaneous abortions.

In a recent observation[1] we measured CA 125 serum levels exceeding 35 U/ml in 16 of 30 (53%) patients with nonviable pregnancies. In age-matched healthy pregnant women of the same gestational week, elevated CA 125 concentrations were found in 8 of 30 (27%). The incidence of elevated CA 125 serum levels in patients with intrauterine fetal death was significantly higher than that in women with viable conceptus ($p < 0.05$, the Wilcoxon U test). Given that intrauterine death is a result of a longer period of fetal impairment, it seems conceivable that CA 125 enters the maternal blood stream before fetal death.

Even though the lead time of the marker CA 125 for

fetal death is not yet defined, the hypothesis that CA 125 might be predictive for threatened abortions can be supported.

Lothar C. Fuith, MD

Department of Obstetrics and Gynecology, University of Innsbruck, Anichstrasse 35, Innsbruck, Austria A-6020

REFERENCE

1. Fuith LC, Marth C, Daxenbichler G. Serum levels of tumor marker CA 125 in viable and nonviable early pregnancy. Gynecol Obstet Invest [In press].

Amniotic endothelin increase during pregnancy

To the Editors: The ability of umbilical endothelial cells to synthesize endothelin in vitro,[1] the presence of endothelin immunoreactivity in umbilical vessels and fetal blood,[2] and the presence of endothelin-specific receptors in human placenta[3] and rat uterus[4] suggest that this potent vasoconstrictor and mitogenic peptide plays important roles in normal and pregnancy and delivery and those with pathologic findings. Endothelin immunoreactivity was shown to be increased in maternal blood during pregnancy[5] and high levels of endothelin immunoreactivity were reported in amniotic fluid at delivery.[6] We now report the kinetics of endothelin immunoreactivity increases in amniotic fluid during normal pregnancies and endothelin immunoreactivity levels in pregnancies complicated by intrauterine growth retardation.

Amniotic fluid (2.5 to 5 ml) was sampled by amniocentesis at two different gestational ages: 16.6 ± 1.6 weeks for prenatal diagnosis of genetic diseases ($n = 27$), and at 37.3 ± 2.2 weeks to verify fetal maturity before elective cesarean section ($n = 8$). All samples were obtained at room temperature (23° C) without anesthesia. Further processing of samples and endothelin detection were performed as previously described.[7]

Endothelin was already detectable in amniotic fluid (18.6 ± 8.9 pg/ml) at 16 weeks' gestation. Its levels increased during the third trimester (106.5 ± 30.9 pg/ml, $p < 0.001$). This value represents 20-fold the levels of endothelin immunoreactivity in normal human plasma (5 ± 1 pg/ml). In three cases complicated with intrauterine growth retardation endothelin immunoreactivity was higher (208.3 ± 109 pg/ml, gestational age 37.6 ± 2.5 weeks).

Although plasma levels of endothelin immunoreactivity are more elevated in pregnant than in nonpregnant women, they do not vary[5] or only slightly increase[6] with gestational age. The fivefold increase of amniotic endothelin immunoreactivity during the last trimester not only supports the hypothesis of a fetoplacental origin of the amniotic peptide but also suggests a controlled mechanism for its access to maternal blood all along the pregnancy. It could be speculated that the elevated concentrations of amniotic endothelin in the late phase of pregnancy may contribute to the uterine contractions and to the umbilical constriction at delivery.

The higher levels of amniotic endothelin immunoreactivity associated with intrauterine growth retardation also raise the possibility of an endothelin contribution to the pathogenesis of those gestational complications related with vasomotor control.

S. Raboni, MD, M.C. Folli, MD, D. Bresciani, MD, A. Bacchi Modena, MD, and A. Merialdi, MD

Istituto di Clinica Ostetrica e Ginecologica, Università degli Studi di Parma, Via Gramsci 14, Parma, Italy 43100

A. Berbinschi, MD, and J.M. Ketelslegers, MD

Unit of Diabetology and Nutrition, University of Louvain, Avenue Hippocrate 54, B-1200 Brussels, Belgium

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1. Clozel M, Fischli W. Human cultured endothelial cells do secrete endothelin-1. J Cardiovasc Pharmacol 1989; 13(suppl 5):S229-31.
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Roster of American obstetric and gynecologic societies*

(appears annually in the January issue)

American Board of Obstetrics and Gynecology, Inc. (1930) President, Albert B. Gerbie. Secretary, James A. Merrill, 4225 Roosevelt Way, N.E., Suite 305, Seattle, WA 98105. Oral examination, Dec. 9-13, 1991.

American Gynecological and Obstetrical Society. President, Kenneth J. Ryan. Secretary, James R. Scott, Department of Obstetrics and Gynecology, 50 N. Medical Dr., Salt Lake City, UT 84132. Meeting, Sept. 5-7, 1991.

Central Association of Obstetricians and Gynecologists. (1929) President, James P. Youngblood. Secretary, William R. Anderson, 421 W. First St., Bloomington, IN 47401. Meeting, Oct. 10-12, 1991.

Pacific Coast Obstetrical and Gynecological Society. (1931) President, Richard N. Bolton. Secretary, Theodore W. Loring, 2607 Harris St., Suite E, Eureka, CA 95501. Meeting, Sept. 9-13, 1991.

Society for Gynecologic Investigation. President, Norman F. Gant, Jr. Secretary, Rogerio A. Lobo, Department of Obstetrics and Gynecology, Women's Hospital, Room 1M2, LAC/USC Medical Center, 1240 N. Mission Road, Los Angeles, CA 90033. Meeting, March 20-23, 1991.

Society of Perinatal Obstetricians. President, Frank Miller. Secretary, Garland Anderson, UTMB/OB-GYN, Galveston, TX 77550. Meetings, Jan. 28–Feb. 2, 1991.

South Atlantic Association of Obstetricians and Gynecologists. (1938) President, M. Tucker Laffitte, Jr. Secretary, W. Glenn Hurt, Obstetrics & Gynecology, Medical College of Virginia, Box 34, Richmond, VA 23298. Meeting, Jan. 27-30, 1991.

American College of Obstetricians and Gynecologists. (1951) President, Ezra C. Davidson, Jr. Secretary, Mary Jo O'Sullivan, 409 12th Street, S.W., Washington, DC 20024-2188. Meeting, May 5-9, 1991.

American Society for Psychosomatic Obstetrics and Gynecology. (1971) President, Kenneth R. Kellner. Secretary, Gail Robinson, 8-231-EN, Toronto General Hospital, 200 Elizabeth St., Toronto, Ontario, Canada M5G 2C4. Meeting, Feb. 27–March 3, 1991.

*Changes, omissions, and corrections must be received by the publisher two months in advance (by November 1) for the Roster. Please address the Journal Editing Department, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318. The number after the society's name is the year of founding. For further information, address the respective secretaries.

Brooklyn Gynecological Society, Inc. President, George Rubino. Secretary, Earl Greenwald, Director, Dept. of OB/GYN, Staten University Island Hospital, 475 Seaview Ave., Staten Island, NY 10305. Meetings, Jan. 6, Feb. 20, March 20, April 17, and May 17, 1991.

Chicago Gynecological Society. (1878) President, John J. Sciarra. Secretary, Allan G. Charles, c/o Dept. OB/GYN, Michael Reese Hospital, 31st & Lakeshore Dr., Chicago, IL 60616. Meetings, third Friday of Jan.-June, 1991.

Cleveland Society of Obstetricians and Gynecologists. President, Gita P. Gidwani. Secretary, Alexander W. Kennedy, Dept. of Gynecology, Cleveland Clinic, A-81, Cleveland, OH 44195. Meetings, Jan. 16, Feb. 23, March 13, and May 22, 1991.

Columbus Obstetric and Gynecologic Society. (1945) President, Carl Krantz. Secretary, Charles A. Caranna, 1215 Brittany Ln., Columbus, OH 43220. Meetings, fourth Wednesday of each month, Sept.-June, 1991.

Greater Hartford Obstetrical and Gynecological Society, Inc. President, Kenneth L. Eckhart, Jr. Secretary, David J. Nochimson, University of Connecticut Health Center, Room C-2165, Farmington, CT 06030. Meetings, Jan. 8 and April 9, 1991.

Memphis and Shelby County Obstetrical and Gynecologic Society. President, Herbert A. Taylor. Secretary, Michael S. Bronstein, 6570 Stage Rd., Suite 160, Memphis, TN 38134. Meetings, second Thursday of each month.

Milwaukee Gynecological Society. (1951) President, Thomas A. Hofbauer. Secretary, Robert H. Herzog, 850 Elm Grove Rd., Elm Grove, WI 53122. Meetings, monthly dinners.

Nassau Obstetrical and Gynecological Society, Inc. President, Milton Eichler. Secretary, Robert Hock, 3051 Long Beach Rd., Oceanside, NY 11572. Meetings, Oct. 29 and Dec. 10, 1991.

New Haven Obstetrical Society. President, Effie Chong. Secretary, Joseph Grasso, Orchard St., New Haven, CT. Meetings, third Tuesday of odd months.

New York Gynecological Society, Inc. President, Victor Rayniak. Secretary, Alfred Williams, 103 Fifth Ave., New York, NY 10003. Meetings, Jan. 23, March 27, May 22, and Oct. 23, 1991.

Northeastern Obstetrical and Gynecological Society. President, Fouad Sattar. Secretary, Robert Dropkin, Executive Park East, Albany, NY 12203. Meetings, Jan. 17, April 18, and Sept. 26, 1991.

San Francisco Gynecological Society. (1929) President, Howard Blanchette. Secretary, Gerald Shefren, 1101 Welch Rd., Palo Alto, CA 94304. Meetings, Jan. 8, Feb. 12, March 12, April 16, and May 14, 1991.

ITEMS

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Common Problems in Obstetric Care, April 3-6, 1991, Westin Maui, Maui, Hawaii. Sponsored by the Departments of Obstetrics and Gynecology, Cornell University Medical College–The New York Hospital and The University of Hawaii Medical Center. For information contact: Colette Carmeris, Course Coordinator, Department of Obstetrics and Gynecology, NYH-CUMC, 525 East 68th St., New York, NY 10021. Tel.: (212) 746-3058.

Issues in Obstetrics and Gynecology, February 25–March 1, 1991, The Hyatt of Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.

3rd Annual Review Course in Obstetrics and Gynaecology, April 30–May 3, 1991, Delta Chelsea Inn, Toronto, Ontario, Canada. AMA category I study credits. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.

Postgraduate Course in Gynecologic and Obstetric Pathology with Clinical Correlation, April 8-12, 1991, Parker House Hotel, Boston, Massachusetts. Fee for course is $650.00 (residents and fellows, $450.00). Address for further information: Department of Continuing Education, Harvard Medical School, 25 Shattuck St., Boston, MA 02115

Sixteenth Annual Update in Obstetrics and Gynecology, Advanced Pelvic Endoscopy, and Physician Workshop on Long Acting Contraception, March 12-17, 1991, Disney's Yacht & Beach Club Convention Center, Lake Buena Vista, Florida. For further information contact Office of Continuing Medical Education, Box J-233, J. Hillis Miller Health Center, Gainesville, FL 32610-0233. Tel.: (904) 392-3143.

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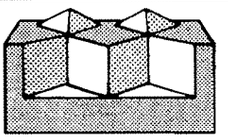
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Candidates for training must, by the effective date of the award, be at a minimum BE in their respective clinical subspecialties and have strong interest in pursuing careers in an academic setting.

Interested individuals should direct applications and inquiries to: Richard L. Berkowitz, M.D., Chairman, Department of Obstetrics, Gynecology & Reproductive Sciences, The Mount Sinai Medical Center, One Gustave L. Levy Place, Box 1170, New York, N.Y. 10029-6574. Telephone: (212) 241-5681.

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John W. Heizer, M.D.,
Medical Director
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Billings, Montana 59107-5100

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Competitive salary, malpractice included. Apply in writing to: Harold E. Fox, M.D., Vice Chairman, Department of OB/GYN, College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032

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Contact:
Richard P. Perkins, M.D.
Professor of Obstetrics and Gynecology
Director of Obstetric Programs
601 N. 30th Street
Omaha, NE 68131

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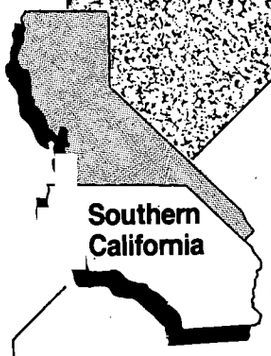
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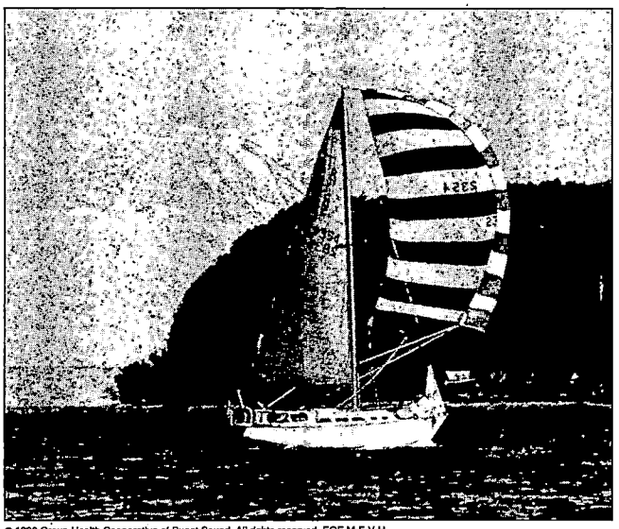
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Assistant Director of Maternal-Fetal Medicine
Department of Obstetrics and Gynecology
Evanston Hospital, Suite 1600
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Evanston, Illinois 60201

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Scott M. Lindblom
Dean Medical Center
1313 Fish Hatchery Road
Madison, WI 53715
Work: (608) 252-8022 Call Collect
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Elko Regional Medical Center
762-14th Street
Elko, Nevada 89801
(702) 738-3111

MATERNAL-FETAL MEDICINE

The Allentown Hospital–Lehigh Valley Hospital Center, an 830-bed tertiary care center in eastern Pennsylvania, is seeking a physician to direct the maternal-fetal service (including Level II ultrasound and antenatal fetal diagnostic procedures) and provide leadership and medical education in maternal-fetal medicine. Applicants should be board-certified or active candidates for certification in maternal-fetal medicine.

TAH–LVHC sponsors a fully accredited OB/GYN Residency Program and is a clinical campus of Hahnemann University in Philadelphia, where the successful candidate will be eligible for faculty appointment. The hospital is a referral center for a population of close to one million people and is a Level III Perinatal Center; of over 3,300 deliveries performed last year, 42% were designated high-risk. The department is supported by a 20-bed Level III NICU and also provides a full range of services to women with subspecialists in both reproductive endocrinology and gynecologic oncology. (Our current maternal-fetal subspecialist, who is our department Chair, will be leaving at the end of the year.)

The hospital is located in Allentown, a prosperous and desirable area with excellent schools and diverse cultural and recreational opportunities, one hour north of Philadelphia and one and one-half hours west of New York City.

Please submit a curriculum vitae, in confidence, to: **Headley S. White, Jr., M.D., Sr. V.P. Medical and Academic Affairs, c/o HealthSearch, 50 College Drive, Suite 325, Allentown, PA 18104, (215) 778-7993.** Equal Opportunity Employer.

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(508) 746-2000,
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Responsibilities include:

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- Direction of Hospital based OB/GYNE Clinic.
- Quality Assurance and Continuing Medical Education for the Division.

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CVs are to be submitted in confidence to: Director of Physician Affairs, 3249 S. Oak Park Ave., Berwyn, IL 60402.

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OB/GYN

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For further details about this exciting practice opportunity, please contact or forward C.V. to: Wendy Tossell, Physician Development Manager, Mercy/St. Charles Hospital, 2600 Navarre Avenue, Oregon, Ohio 43616 (419) 698-7645.

OBSTETRICIANS & GYNECOLOGISTS

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Obstetrics and Gynecology
Maternal/Fetal Medicine
4755 Ogletown-Stanton Road
P.O. Box 6001
Newark, DE 19718

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Bronson Healthcare Group
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800-594-9022 or
616-341-6331 collect

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Our hospital is located 25 miles from Hartford, Connecticut, in an area that offers exceptional residential, cultural, recreational, and educational opportunities.

Send Curriculum Vitae to Search Committee c/o Susan Menichetti, Vice President, Administration, Middlesex Hospital, 28 Crescent Street, Middletown, CT 06457.

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If interested, call me at (702) 877-8638 or send your Curriculum Vitae to: Jerry Reeves, M.D., President, Southwest Medical Associates, P.O. Box 15645, Las Vegas, NV 89114.

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The Department of Obstetrics and Gynecology at Truman Medical Center-University of Missouri at Kansas City seeks a board certified/board eligible sub-specialist to direct the Division of Reproductive Endocrinology. This position entails clinical and didactic teaching of both residents and medical students, participation in current projects and development of new areas of research. The candidate may assume a large office-based practice of subspecialty patients which offers a full range of assisted reproductive technologies. The hospital offers a very generous benefit package. Salary, hospital and university appointments will be commensurate with experience and qualifications.

Please send resume to:

Marilyn R. Richardson, M.D.
Director, Division of
Reproductive Endocrinology
Truman Medical Center
2301 Holmes Street
Kansas City, Missouri 64108

OB/GYN CHIEF

The Department of Gynecology and Obstetrics at The Union Memorial Hospital is currently accepting applications for the position of Chief of Gynecology and Obstetrics and director of a fully accredited residency training program. Union Memorial is a 350 bed teaching hospital in Baltimore, Maryland. Applicants must be board certified in OB/GYN with subspecialty boards preferred, have administrative, clinical, teaching, and research experience. For confidential consideration, please send CV to:

Marcella Roenneburg, M.D.
The Union Memorial Hospital
201 E. University Parkway
Baltimore, Maryland 21218

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Assistant Professor
Obstetrics, Gynecology and Reproductive Sciences
Obstetrician-Gynecologist

The University of California, San Francisco, Department of Obstetrics, Gynecology and Reproductive Sciences, is seeking a full time faculty member at the Assistant Professor level.

Applicants for this position should be well prepared clinically. Responsibilities will include teaching and patient care activities in general obstetrics and gynecology.

Please send curriculum vitae to:

Nancy Milliken, M.D.
Chair, Search Committee
University of California, San Francisco
San Franciso, CA 94143-0346

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MATERNAL-FETAL MEDICINE POSITION
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
UNIVERSITY OF ILLINOIS HOSPITAL
CHICAGO, ILLINOIS

The Department of Obstetrics and Gynecology at the University of Illinois, Chicago, has an unexpected opening for a first year Fellowship position in Maternal-Fetal Medicine beginning, July 1, 1991. This is a two-year accredited Fellowship program in which both clinical and research training are emphasized.

The Fellowship program at the University of Illinois Hospital and the affiliated Michael Reese Hospital is the largest perinatal clinical service in the City of Chicago, with more than 7000 deliveries per year, approximately 50% of which are high risk. There is an active prenatal genetic program including CVS. A large baboon colony is available for basic perinatal research. In addition to an active MD and clinical research faculty, the department employs basic science Ph.D. faculty. Residents who are interested in applying for the Fellowship should contact Andre Bieniarz, M.D., Director, Maternal-Fetal Medicine Fellowship, Department of Obstetrics and Gynecology, University of Illinois College of Medicine, 840 S. Wood Street, Chicago, IL 60612.

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OBSTETRICIAN/GYNECOLOGIST
University of Vermont
College of Medicine

The Department of Obstetrics and Gynecology, University of Vermont College of Medicine is seeking an Assistant Professor who is board certified or eligible in General Obstetrics and Gynecology. Candidates should demonstrate interest and ability in teaching and patient care. Salary is competitive and commensurate with experience.

Applications will be accepted until position is filled. Respond with curriculum vitae to:

Daniel H. Riddick, M.D., Ph.D.
Professor and Chairman
Department of Obstetrics and Gynecology
Medical Center Hospital of Vermont
Burlington, VT 05401

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Chair of Obstetrics & Gynecology

The University of Ottawa invites applications for the position of Chairperson of the University Department of Obstetrics & Gynecology.

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The Department is responsible for directing both undergraduate teaching and postgraduate programs in Obstetrics & Gynecology in the Ottawa Civic Hospital and Ottawa General Hospital. The successful candidate will hold a joint appointment as Chair of the University Department of Obstetrics & Gynecology and Head of one of the affiliated teaching hospitals.

The Faculty is seeking an individual who will hold a fulltime University appointment. Salary and fringe benefits are commensurate with qualifications and experience, and are in accordance with existing scales at the University of Ottawa.

Priority will be given to Canadian citizens and permanent residents of Canada in accordance with Canadian immigration requirements. Employment equity is University policy.

A working knowledge of both English and French is desirable and a commitment to support bilingualism with the Faculty is essential.

Applicants are requested to forward their Curriculum Vitae and the names of three referees **prior to** February 15, 1991 to:

John F. Seely, M.D.
Dean, Faculty of Medicine
University of Ottawa
451 Smyth Road
Ottawa, Ontario
K1H 8M5

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- Board eligibility or certification in gynecologic oncology required.
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Curriculum Vitae should be sent to:

Garland D. Anderson, M.D.
Jennie Sealy Smith Professor & Chairman
Department of Obstetrics & Gynecology
The University of Texas Medical Branch
Galveston, Texas 77550-2776

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Hanns C. Haesslein, M.D.
Perinatal Associates
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5301 F. Street
Sacramento, California 95819

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NDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

ONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following onditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophle- itis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected arcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-depen- lent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice vith prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including nyocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the isk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The isk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long erm use of the oral contraceptives with lower formulations of both estrogens and progestogens remains o be determined. Throughout this brief summary epidemiological studies reported are of two types: etrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert.**

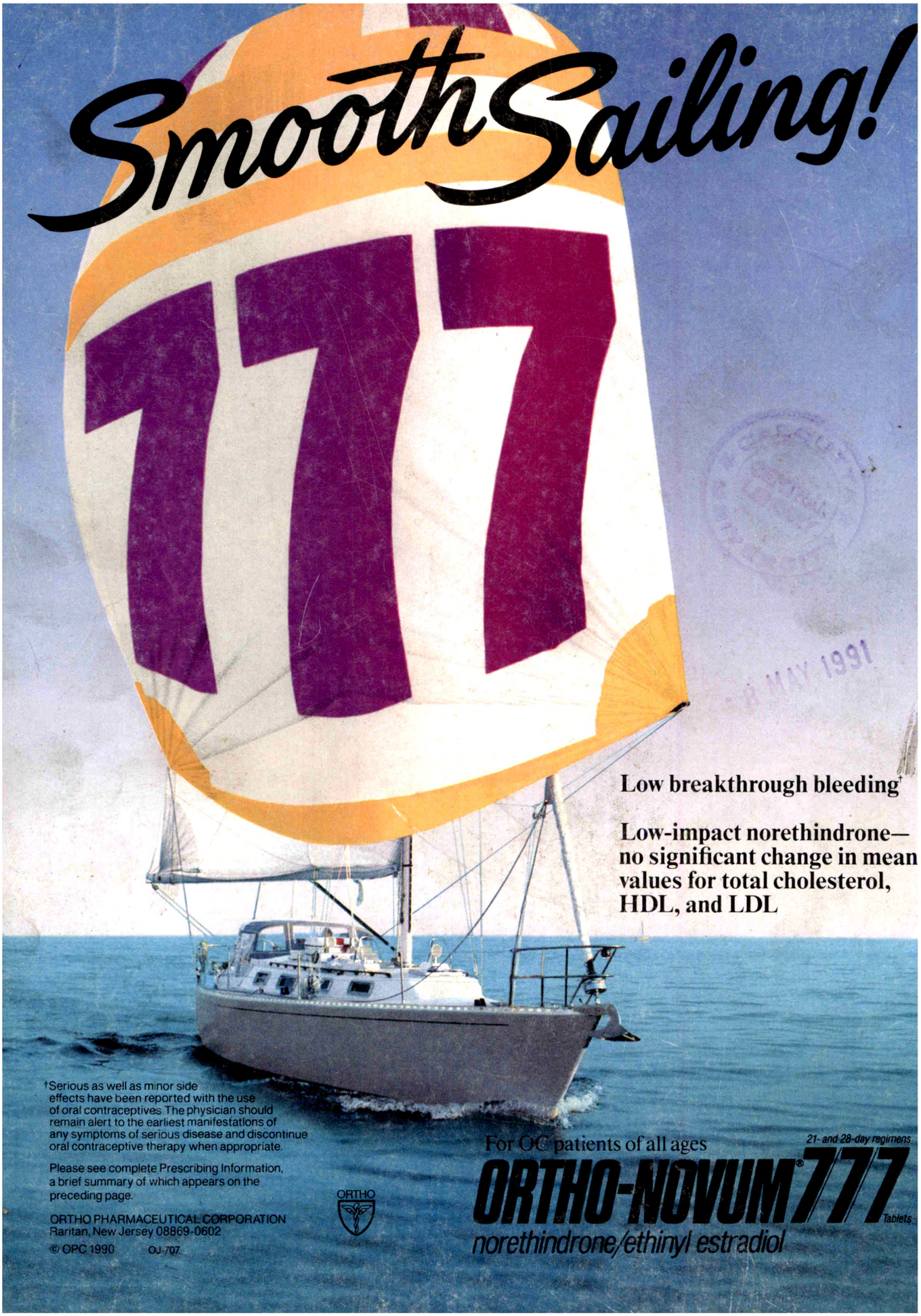
ADVERSE REACTIONS: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis.

OVERDOSAGE: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Revised 9/89

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Raritan, NJ 08869-0602

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Smooth Sailing!
777
Low breakthrough bleeding†
Low-impact norethindrone—no significant change in mean values for total cholesterol, HDL, and LDL
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Please see complete Prescribing Information, a brief summary of which appears on the preceding page.
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For OC patients of all ages
21- and 28-day regimens
ORTHO-NOVUM® 777 Tablets
norethindrone/ethinyl estradiol

February 1991
volume 164, *number* 2

American Journal of OBSTETRICS AND GYNECOLOGY

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Going.

Going.

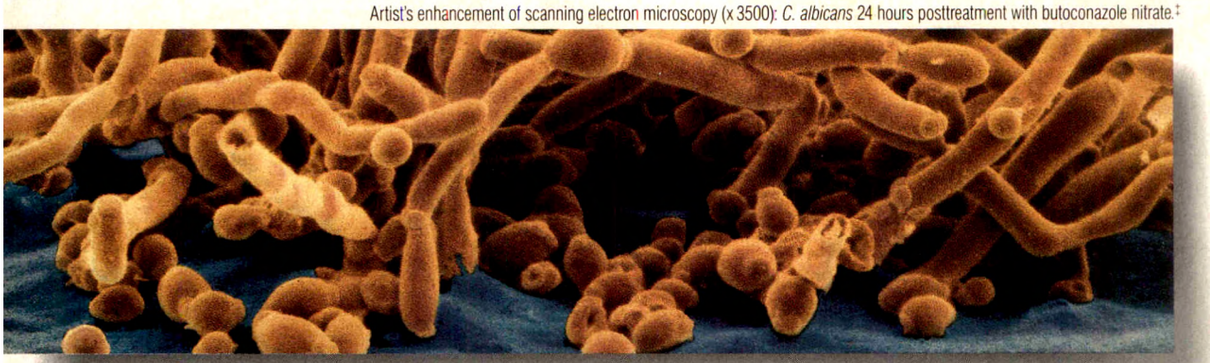
Gone.

***In vulvovaginal candidiasis,* the proven efficacy of 3-day therapy* with FEMSTAT Prefill means a microbiological cure in 95% of patients.[1†]**

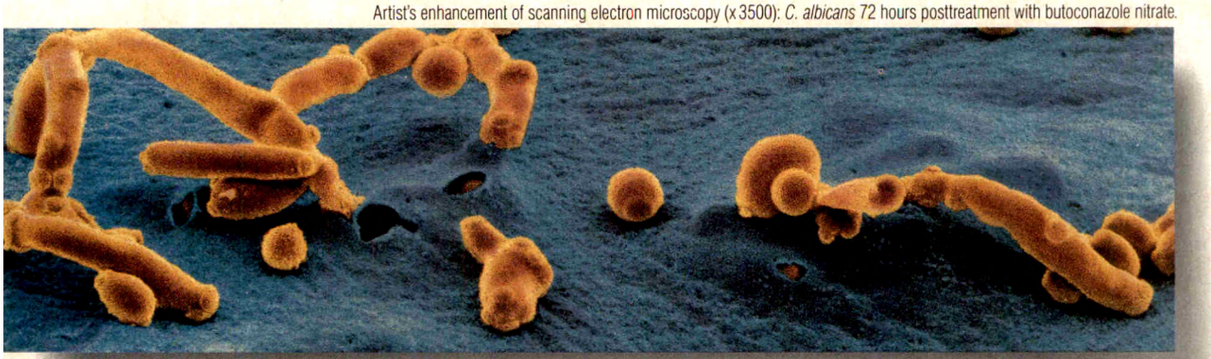


1. Droegemueller W, Adamson DG, Brown D, et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 1984;64:530-534. *In pregnant patients (second and third trimesters only), therapy should be extended to six days. †Microbiological cure in 95% of patients (n = 126) eight days following FEMSTAT 3-day therapy. ‡ Micrographs on file, Syntex Laboratories, Inc. Please see next page for brief summary of prescribing information. © 1990 Syntex Laboratories, Inc. 813-H2-166-90

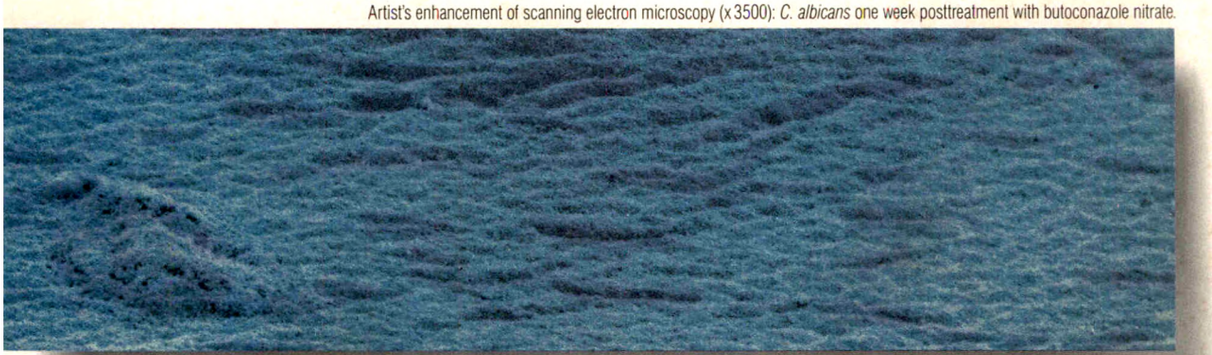
Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 24 hours posttreatment with butoconazole nitrate.‡



Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 72 hours posttreatment with butoconazole nitrate.



Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* one week posttreatment with butoconazole nitrate.



FEMSTAT® PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%

So effective, it works with 3-day therapy.



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PALO ALTO, CA 94304

Committed to the OB/GYN and women's healthcare

FEMSTAT PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%



BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

CURRENT PROBLEMS IN OBSTETRICS, GYNECOLOGY AND FERTILITY

Editor-in-Chief: Robert L. Barbieri, MD
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THE GOOD THINGS
SHE DOES FOR HERSELF,
SHE STAYS WITH...
She stays with TRIPHASIL®
for the same reasons
she started with it:
Minimal breakthrough
bleeding from Day 1
Low estrogen dose
Low incidence of common
OC side effects*
Triphasil®
Levonorgestrel- and ethinyl estradiol tablets—
Triphasic regimen 21- and 28-day regimens
THE OC TO START WITH
BECAUSE SHE'LL STAY WITH IT
*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. See prescribing information.
See brief summary on adjacent page.
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Triphasil®

Levonorgestrel and ethinyl estradiol tablets—
Triphasic regimen 21- and 28-day regimens

THE OC TO START WITH, BECAUSE SHE'LL STAY WITH IT

IN BRIEF:
TRIPHASIL® — 6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen) — Triphasic regimen.

Indications and Usage — TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications — OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thrombo-embolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners pre-scribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS — In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI) — An increased risk of MI associated with the use of OC's has been reported, con-firming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE — In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES — A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34 — 5/100,000; ages 35-44 — 33/100,000; ages 45-49 — 140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS — Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions* — There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma* — Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors* — Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring* — Use of female sex hormones — both estrogenic and progestational agents — during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease* — Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects* — Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure* — Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache* — Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities* — Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy* — Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding* — OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions — GENERAL — 1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear. 2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related. 4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined. 8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's. a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient — See Patient Package Labeling.

Drug Interactions — Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis — See Warnings section for information on carcinogenesis.

Pregnancy — Category X. See Contraindications, Warnings.

Nursing Mothers — See Warnings.

Adverse Reactions — An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose — Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration — For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.

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Worldwide Leadership in Female Healthcare

Mosby Year Book

American Journal of Obstetrics and Gynecology

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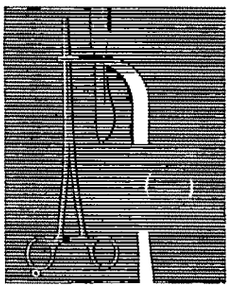
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In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

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Count on

CEFOTAN® IM/IV

(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary tract infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).
Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli.*
Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli.*
Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).
Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).
Bone and joint infections caused by *Staphylococcus aureus.**
*Efficacy for this organism in this organ system was studied in fewer than ten infections.
Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.
In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.
NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).
The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.
If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.
Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.
Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.
Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.

PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.
In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.
CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.
INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.
DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.
As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.
CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.
Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.
Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.
USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.
USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.
PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).
Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).
Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).
Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).
Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.
IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.
Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.
When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

Females: 0.9 × value for males

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.
1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

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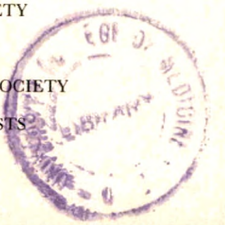
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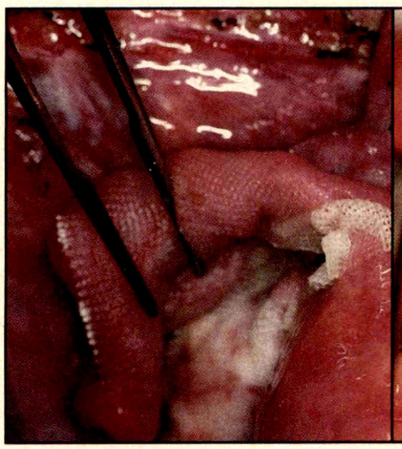
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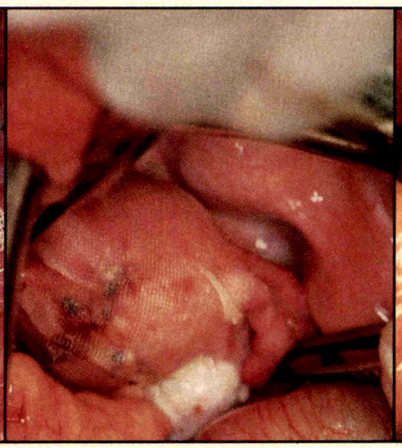


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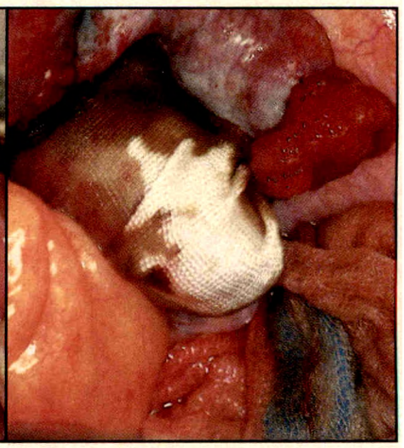
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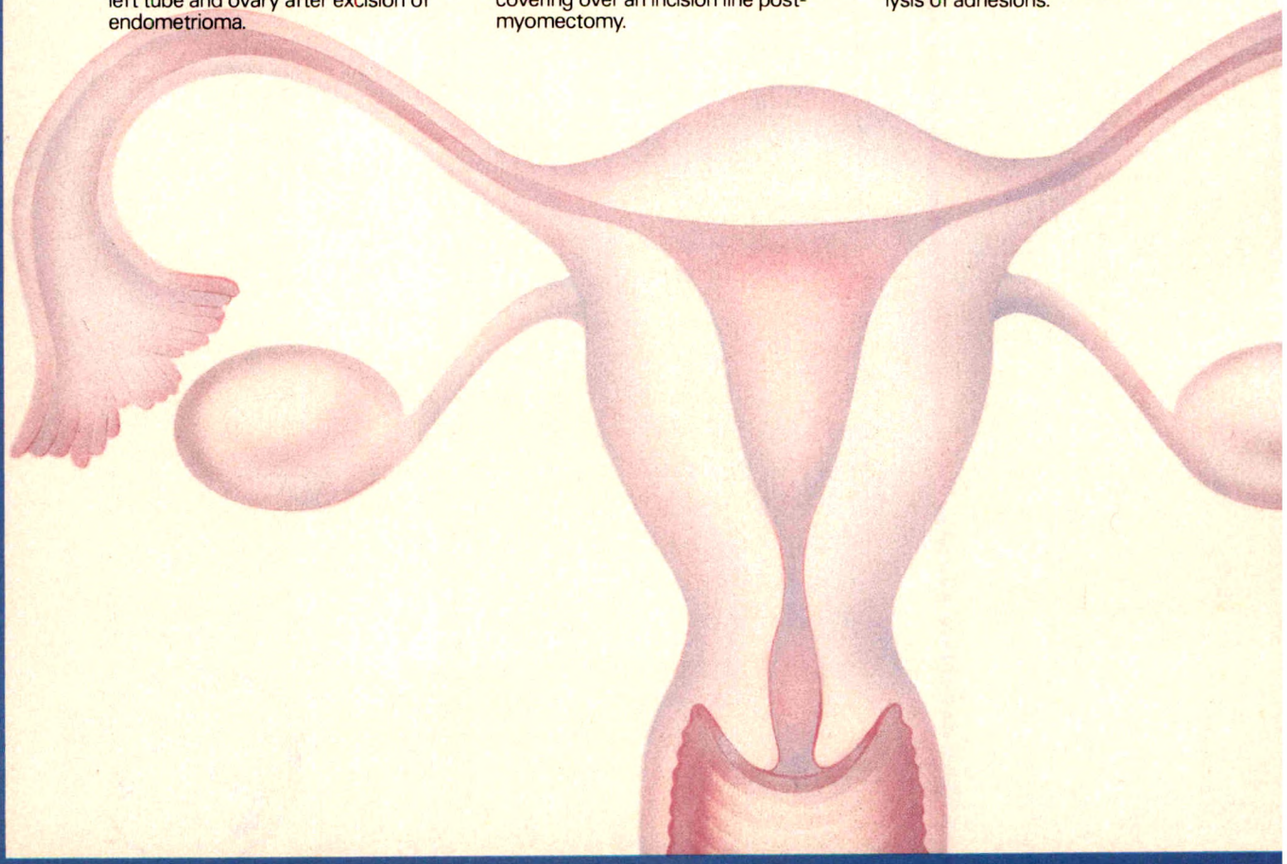
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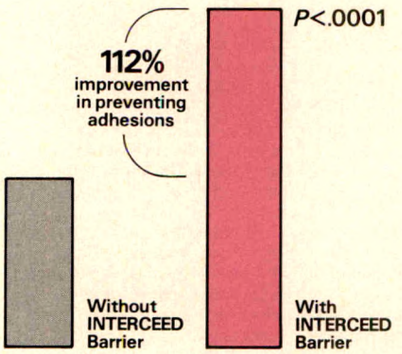
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1. Data on file, Johnson & Johnson Medical, Inc.
2. Diamond MP, Cunningham T, Linsky CB, et al. Laparoscopic application of Interceed (TC7) in the Pig. *J Gynecol Surg.* 1989;5:145-148.

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Carcinogenesis, Mutagenesis, Impairment of Fertility: Acyclovir was tested in lifetime bioassays in rats and mice at single daily doses of 50, 150 and 450 mg/kg/day given by gavage. These studies showed no statistically significant difference in the incidence of benign and malignant tumors produced in drug-treated as compared to control animals, nor did acyclovir induce the occurrence of tumors earlier in drug-treated animals as compared to controls. In 2 *in vitro* cell transformation assays, used to provide preliminary assessment of potential oncogenicity in advance of these more definitive lifetime bioassays in rodents, conflicting results were obtained. Acyclovir was positive at the highest dose used in one system and the resulting morphologically transformed cells formed tumors when inoculated into immunosuppressed, syngeneic, weanling mice. Acyclovir was negative in another transformation system.

No chromosome damage was observed at maximum tolerated parenteral doses of 100 mg/kg acyclovir in rats or Chinese hamsters; higher doses of 500 and 1000 mg/kg were clastogenic in Chinese hamsters. In addition, no activity was found in a dominant lethal study in mice. In 9 of 11 microbial and mammalian cell assays, no evidence of mutagenicity was observed. In 2 mammalian cell assays (human lymphocytes and L5178Y mouse lymphoma cells *in vitro*), positive response for mutagenicity and chromosomal damage occurred, but only at concentrations at least 1000 times the plasma levels achieved in man following topical application.

Acyclovir does not impair fertility or reproduction in mice at oral doses up to 450 mg/kg/day or in rats at subcutaneous doses up to 25 mg/kg/day. In rabbits given a high dose of acyclovir (50 mg/kg/day, s.c.), there was a statistically significant decrease in implantation efficiency.

Pregnancy: *Teratogenic Effects.* Pregnancy Category C. Acyclovir was not teratogenic in the mouse (450 mg/kg/day, p.o.), rabbit (50 mg/kg/day, s.c. and i.v.) or in standard tests in the rat (50 mg/kg/day, s.c.). In a non-standard test in rats, fetal abnormalities, such as head and tail anomalies, were observed following subcutaneous administration of acyclovir at very high doses associated with toxicity to the maternal rat. The clinical relevance of these findings is uncertain.[2] There are no adequate and well-controlled studies in pregnant women. Acyclovir should not be used during pregnancy unless the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Zovirax is administered to a nursing woman.

ADVERSE REACTIONS: Because ulcerated genital lesions are characteristically tender and sensitive to any contact or manipulation, patients may experience discomfort upon application of ointment. In the controlled clinical trials, mild pain (including transient burning and stinging) was reported by 103 (28.3%) of 364 patients treated with acyclovir and by 115 (31.1%) of 370 patients treated with placebo; treatment was discontinued in 2 of these patients. Other local reactions among acyclovir-treated patients included pruritus in 15 (4.1%), rash in 1 (0.3%) and vulvitis in 1 (0.3%). Among the placebo-treated patients, pruritus was reported by 17 (4.6%) and rash by 1 (0.3%).

In all studies, there was no significant difference between the drug and placebo group in the rate or type of reported adverse reactions nor were there any differences in abnormal clinical laboratory findings.

OVERDOSAGE: Overdosage by topical application of Zovirax Ointment 5% is unlikely because of limited transcutaneous absorption (see Clinical Pharmacology).

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REFERENCES

1. Naib ZM et al. *Cancer Res* 33:1452-1463, 1973.
2. Stahlmann R, Klug S, Lewandowski C, et al. *Infection:* 15(4): 261-262, 1987.

U.S. Patent No. 4199574

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CLINICAL SECTION

Clinical Opinion

Epidemiologic characteristics of preterm delivery: Etiologic heterogeneity

David A. Savitz, PhD, Cheryl A. Blackmore, MPH, and John M. Thorp, MD

Chapel Hill, North Carolina

Preterm delivery (<37 weeks completed gestation) is known to result from diverse etiologic pathways, which can be grouped into idiopathic preterm labor, preterm premature rupture of the membranes, and medical complications. Data from publications providing sufficient detail to subdivide preterm delivery cases into these groups were tabulated. In spite of inconsistent terminology and incomplete reporting, patterns were identified. Black women have a markedly higher risk of preterm delivery, which is especially pronounced for preterm premature rupture of the membranes. Idiopathic preterm labor is predominant in lower-risk, white populations. These observations encourage consideration of subcategories of preterm delivery in studies of etiology and prevention. (AM J OBSTET GYNECOL 1991;164:467-71.)

Key words: Preterm delivery, preterm labor, premature rupture of the membranes, epidemiology

Early delivery is known to be related to increased infant morbidity and mortality, in both developed and developing countries.[1] Most studies of the causes and consequences of preterm delivery rely exclusively on gestational age of the infant, with little attention to the reasons for which the delivery occurred early. Studies that are based on vital records,[2, 3] for example, cannot evaluate specific causal pathways leading to early delivery since the information typically contained in birth certificates is inadequate for that purpose. Nonetheless, it is recognized that there are many different reasons for which the period of gestation may be undesirably curtailed.

The most common mechanisms involve idiopathic preterm labor or preterm premature rupture of the membranes, defined by whether labor or membrane rupture precipitates the sequence of events that culminate in delivery. In addition, there are numerous medical indications for early delivery such as fetal distress, maternal bleeding, or severe preeclampsia. The diversity of processes capable of resulting in preterm delivery would suggest that efforts to identify etiologic agents and ultimately methods of prevention consider each of those processes separately. If, for example, an exposure is related exclusively to idiopathic preterm labor, the observed association with preterm deliveries in the aggregate will be attenuated by a form of misclassification.[4] Thus studying more homogeneous entities should allow for the identification of quantitatively stronger determinants. Furthermore, preterm delivery prevention programs are often actually designed to recognize and intervene early if preterm labor begins, with success determined in part by the relative contribution of preterm labor to all preterm deliveries.[5-8] Finally, little attention has been paid to the possibility that the clinical consequences of prematurity could vary by the causal mechanism; i.e., whether the cause of prematurity or only the fact that it has occurred determines the sequelae for the infant.

Some investigators have addressed the heterogeneity

From the Department of Epidemiology, School of Public Health, and the Division of Maternal and Fetal Medicine, Department of Obstetrics and Gynecology, School of Medicine, University of North Carolina.

Reprint requests: David A. Savitz, PhD, Department of Epidemiology, CB No. 7400, School of Public Health, University of North Carolina, Chapel Hill, NC 27599-7400.

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in preterm delivery. Daikoku et al.[9] and Berkowitz[10] restricted their studies to noniatrogenic causes, principally preterm labor and preterm premature rupture of the membranes. In the context of a prematurity prevention program, Meis et al.[7] have provided the most sophisticated analysis of etiologic pathways among different racial and socioeconomic groups. A few other investigators, cited below, have provided sufficient detail to assess the relative or absolute frequency of preterm delivery resulting from different processes. As a prelude to studies of the specific entities of interest (e.g., preterm labor) and to appreciate the patterns of variation across populations, studies that permitted an assessment of patterns of preterm delivery were compiled for review.

Methods

Publications on preterm delivery that contained any data to subdivide cases by cause were sought. Varying definitions of preterm delivery were considered, with deviations from the standard definition of "<37 weeks completed gestation" noted, e.g., preterm among low-birth-weight infants. The following categories of preterm delivery were tabulated: (1) idiopathic preterm labor—labor starting without apparent reason before rupture of the membranes, resulting in preterm delivery; (2) preterm premature rupture of the membranes—spontaneous rupture of the fetal membranes at any time before the onset of labor resulting in preterm delivery; (3) iatrogenic—medically induced preterm delivery typically caused by conditions such as severe preeclampsia, maternal disease, or bleeding. Rarer causes such as trauma were not tabulated separately and were grouped with iatrogenic cases.

Characteristics of the study populations that were tabulated included the source [clinics, referral hospital(s), community census], geographic area, years, racial mix of patients, and socioeconomic characteristics of the patients. As available, the numerator and denominator for deriving the total preterm delivery proportion were obtained, with estimates of the subtypes of preterm delivery as a fraction of all cases and as a fraction of all births tabulated. Data often were not presented in the desired manner, but published results were reanalyzed, when possible, to derive the proportions of interest.

Results

The inconsistency in terminology across studies is striking. In some instances preterm births were restricted to those of a particular weight. Premature rupture of the membranes was often analyzed without stratification by gestational age. Articles that provided cross-classification of the data by several attributes were most informative, allowing reanalysis for the purposes of this review.

Estimates of the frequency of subtypes of preterm delivery were available from 16 populations, on the basis of 12 publications during the 1980s[7-18] (Table I). Most reports were based on case series from large referral hospitals, with one study of a geographically defined population[18] and one study subdivided into private and public clinics.[7] This simple division by provider yields useful information on social class and could easily have been incorporated into some of the other studies.

The studies were concentrated in the United States, with most covering urban areas, and two studies were from Scandinavia. Deliveries as far back as 1951 were included with the possibility of differences among studies as a result of secular trends in the causes of preterm delivery. Both white and black populations have been studied, as well as a range of social classes. For the purposes of describing the patterns sought in this paper, greater detail on the sources and social characteristics of patients would have been desirable. In many reports the source was defined only as the hospital, with no information on attributes of the population served.

Table II describes the patterns of preterm delivery across these populations. The study sizes vary markedly, ranging from 45[7] to 11,832[14] preterm cases. The overall risk of preterm delivery also varied markedly, from 4.4%[15] to 21.5%.[13] There is a clear pattern according to race: Black populations are at much higher risk of preterm delivery, with risks ranging from 10.8% to 17.8%. The highest rate (21.5%) was reported from the highly selected population that participated in the Collaborative Perinatal Project.[13] Lower rates were found in white and biracial populations, with some variation because of more restrictive definitions of preterm delivery (e.g., noniatrogenic cases,[10] preterm and low birth weight).[7, 10]

The distribution of subtypes of preterm delivery also varied across populations, with a tendency for low-risk populations to have the highest proportions of idiopathic preterm labor. Only the Finnish population[18] and a private clinic white population[7] had >50% of all preterm deliveries caused by preterm labor, with more than half of the noniatrogenic cases in Berkowitz's biracial population attributed to preterm labor.[10] The lowest proportions (under 30%) were reported in a public clinic black population[7] and from a referral hospital in St. Louis.[17]

Preterm premature rupture of the membranes varied from 7.1%[18] to 51.2%[7] of all preterm deliveries, with some of the range resulting from differing definitions of the total case group. Meis et al.[7] found that a greater proportion of preterm deliveries were due to preterm premature rupture of the membranes among blacks

Table I. Studies with subclassification of preterm delivery: Description of study populations

Authors and year	*Reference No.*	*Source*	*Geographic area*	*Years*	*Race*	*Social class*
Johnson et al., 1981	11	Referral hospital	Baltimore	1951-1964	White	Middle
Johnson et al., 1981	11	Referral hospital	Baltimore	1951-1964	Black	Lower
Naeye and Peters, 1980	12	Referral hospitals	U.S. (selected areas)	1959-1966	White and black	Lower and middle
Naeye, 1982	13	Referral hospitals	U.S. (selected areas)	1959-1966	White and black	Lower and middle
Kaltreider and Kohl, 1980	14	Not stated	U.S. and Canada	1970-1976	White and black	Mixed
Miller and Jekel, 1989	15	Referral hospital	Kansas	1975-1978	White	Mixed
Evaldson et al., 1980	16	Referral hospital	Sweden	1976-1977	White	Middle
Daikoku et al., 1982	9	Referral hospital	Baltimore	1976-1977	Not stated	Not stated
Johnson et al., 1981	11	Referral hospital	Baltimore	1976-1979	White and black	Mixed
Arias and Tomich, 1982	17	Referral hospital	St. Louis	1978-1979	Not stated	Not stated
Berkowitz, 1985	10	Referral hospital	New Haven, Conn.	1977-1978	White and black	Lower and middle
Piekkala et al., 1986	18	Population-based	Turku, Finland	1981-1982	Not stated	Not stated
Meis et al, 1987	7	Private clinic	North Carolina	1983-1984	White	Upper and middle
Meis et al., 1987	7	Public clinic	North Carolina	1983-1984	White	Lower
Meis et al., 1987	7	Public clinic	North Carolina	1983-1984	Black	Lower
Main et al., 1985	8	Referral hospital	Philadelphia	1983-1984	Black	Lower

and public (lower social class) patients as compared with whites and private (higher social class) patients, whereas this was not evident in the study of Johnson et al.[11] or in the literature overall. The medical causes of preterm delivery showed less variation. Proportions ranged only from 18.7% to 28.8%, with the exception of Arias and Tomich,[17] who included multiple gestations in this category, in contrast to other researchers who restricted their study populations to singleton births.

The absolute frequency of the subtypes of preterm delivery is a product of the risk of preterm delivery and the proportion associated with the specific cause. Preterm labor was most frequent in the (undefined) population served by The Johns Hopkins University Hospital,[9] a black population in Philadelphia,[8] and, somewhat surprisingly, in Finland.[18] Preterm premature rupture of the membranes showed a much clearer pattern of high risk for blacks, with risks of ≥5% observed in three studies focused on black populations,[7,8,11] as well as in the Collaborative Perinatal Project data[13] and in the population served by The Johns Hopkins University Hospital.[9] Notably low risks were found for white populations in Kansas,[15] North Carolina,[7] Finland,[18] and Sweden[16] and in the population of the Obstetrical Statistical Cooperative.[14] Unusually high frequencies of medically induced preterm deliveries were reported by Arias and Tomich[17] and Main et al.[8]

Comment

In spite of the well-recognized differences in the etiologic pathways resulting in preterm delivery, there has been little epidemiologic research to discriminate among them. To our knowledge, there are no studies focused on identifying risk factors for case groups defined by the mechanism of preterm delivery. In some studies of preterm delivery or even low birth weight, there would be the opportunity to assess determinants of case subgroups if researchers organized the available data appropriately.

Some studies of etiology have focused on specific associations, e.g., sexually transmitted diseases and premature rupture of the membranes.[19] However, most studies that address causes of preterm delivery do so without separating causal pathways.[20,21] The data summarized here suggest that there may be different risks for the different pathways of preterm delivery on the basis of racial and socioeconomic differences. The overall pattern reported by Meis et al.[7] is corroborated to some extent in other studies. White patients and those of higher social classes tend to have proportionately greater numbers of cases of preterm labor whereas

Table II. Patterns of preterm delivery: Relative and absolute frequency of subtypes

Author and year	Reference	Preterm births		Percent of preterm births			Percent of all births		
		No.	%	Preterm labor	Preterm-PROM	Medical	Preterm labor	Preterm-PROM	Medical
Johnson et al., 1981*	11	1,601	7.7	NA	29.7	NA	NA	2.3	NA
Johnson et al., 1981†	11	3,395	17.8	NA	28.7	NA	NA	5.1	NA
Naeye and Peters, 1980	12	6,613	NA	NA	31.9	NA	NA	NA	NA
Naeye, 1982	13	1,993	21.5	NA	33.1	NA	NA	7.1	NA
Kaltreider and Kohl, 1980‡	14	11,832	4.9	NA	34.3	NA	NA	1.7	NA
Miller and Jekel, 1989	15	67	4.4	NA	35.8	NA	NA	1.6	NA
Evaldson et al., 1980	16	NA	NA	NA	NA	NA	NA	0.9	NA
Daikoku et al., 1982§	9	477	17.0	57.4	42.6	§	9.7	7.2	§
Johnson et al., 1981‖	11	1,174	NA	NA	38.1	NA	NA	NA	NA
Arias and Tomich, 1982‡	17	355	8.6	29.9	34.9	35.2	2.6	3.0	3.0
Berkowitz, 1985§	10	175	4.8	57.7	42.3	§	2.8	2.0	§
Piekkala et al., 1986	18	351	6.6	64.1	7.1	28.8	4.2	0.5	1.9
Meis et al., 1987‡,¶	7	75	5.7	54.6	26.7	18.7	3.1	1.5	1.1
Meis et al., 1987†,#	7	45	6.1	44.4	35.6	20.0	2.7	2.2	1.2
Meis et al., 1987‡,**	7	86	10.8	27.9	51.2	20.9	3.0	5.6	2.3
Main et al., 1985	8	58	15.4	37.9	37.9	24.1	5.9	5.9	3.7

PROM, Premature rupture of the membranes; *NA*, not available.

*White patients, 1951 to 1964.

†Black patients, 1951 to 1964.

‡Preterm among low-birth-weight (<2500 gm) infants.

§Preterm cases not caused by premature rupture of membranes or spontaneous labor excluded.

‖White and black patients, 1976 to 1979.

¶Private clinic.

#Public clinic white patients.

**Public clinic black patients.

black patients and those of lower social classes tend to have more instances of premature rupture of the membranes. Given the overall higher risk of preterm delivery among black women, the absolute (as opposed to proportional) increase of premature rupture of the membranes is more notable.

These suggestions of heterogeneity encourage studies designed to elucidate the causes and assess the preventability of specific pathways for preterm delivery. Etiologic studies focused on homogeneous end points are much more likely to yield strong associations than are studies of heterogeneous end points. Nonetheless, it is possible that some of the apparently diverse pathways through which preterm delivery occurs may actually be manifestations of the same underlying pathologic condition. For example, certain infections appear to affect both preterm labor and preterm premature rupture of membranes.[22, 23] Only by disaggregating the cases into subgroups can that possibility be addressed.

The intense interest in preterm delivery prevention programs provides another strong incentive to evaluate more thoroughly the potential benefits of such interventions. These programs are largely based on identifying and responding to early signs of preterm labor,[7, 8] so that their success is determined in part by the extent to which preterm labor is the dominant cause of preterm delivery. Other obstetric practices and interventions such as routine vaginal examination are also under scrutiny for their potential beneficial or adverse consequences on the risk of preterm premature rup-

ture of the membranes.[24] In this case there may be a tradeoff in the risks and benefits with a potential advantage for detecting and treating cervical incompetence and a potential risk of introducing infection and precipitating membrane rupture or labor. The relative frequency of those possible outcomes must be considered in drawing conclusions, with the overall balance dependent in part on the characteristics of the population under study.

In spite of the limited quantity and quality of information on subtypes of preterm delivery, these results encourage further research. Investigators need to define more carefully the outcomes of interest in studies of both cause and interventions. Dividing preterm delivery into more homogeneous and thus tractable components is probably essential to progress toward understanding and prevention of this common problem.

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Addendum in proof

A recent article reports on a study focused on the cause of preterm premature rupture of the membranes (Harger JH, Hsing AW, Tuomala RE, et al. Risk factors for preterm premature rupture of fetal membranes: a multicenter case-control study. AM J OBSTET GYNECOL 1990;163:130-7).

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Menstrual mythology and sources of information about menstruation

David C. Cumming, MB, ChB, Ceinwen E. Cumming, PhD, and Dianne K. Kieren, PhD
Edmonton, Alberta, Canada

Surveys suggest that, despite extensive scientific knowledge of the biologic rhythms and physical changes associated with reproduction and despite the availability of excellent educational material about sexuality including menstruation, young peoples' knowledge of reproductive physiology is inadequate. Superstitions, illogical beliefs, and misinterpretations are more common than accurate understanding. The present article reviews menstrual mythology and describes sources of information for young people. Family and specialist physicians as well as educators in school or in the community need to recognize the limitations of the present methods of sexuality education, must realize that understanding of reproductive processes may be very incomplete, and should be prepared to work cooperatively with informal sources of information (peers, parents, and commercial companies). In addition, menstrual education needs to move away from the focus on hygienic management to that of healthy sexuality and acceptance of self. (AM J OBSTET GYNECOL 1991;164:472-6.)

Key words: Menstruation, education, physician's role

Formal teaching of reproductive physiology and sexuality to young people involves more than the provision of facts; it must be provided within a clear understanding of the social and physical developmental needs of young people as well as the sociologic, ethical, moral, and religious contexts within which these needs are met. Information about reproductive function and sexuality is available to young people from a number of formal and informal sources. The ability to deal with the problems and misconceptions of menstruation requires an understanding of the ways in which sexuality information is provided to young people by formal educational sources and from commercial companies, parents, and peers.

Physicians learn reproductive physiology in what it is hoped are optimal circumstances and therefore may not be aware that the quality of information available to young people is variable and leads to the persistence of menstrual mythology. We have a special interest in promoting our patients' understanding of normal reproductive physiology, because women with accurate knowledge are less likely to misinterpret the significance of symptoms and more likely to be aware of when events in the menstrual cycle are abnormal. We are formal educators in the sense of knowledge, but our opportunities to educate are informal and we use the "teachable moment." Physicians are criticized because of a perceived discomfort in discussing sexuality and because of problems of application and interpretation of our factual knowledge within a human interpersonal context.

Our purpose in this article is to review myths and misinterpretations related to menstruation, sources of information currently available for menstrual education, and the implications for physicians and others involved in menstrual education programs in family, school, and community contexts. By understanding and supporting efforts to provide a high standard of education in reproduction and sexuality, we may improve the quality of life for our patients and diminish functional reproductive problems.

From the Departments of Obstetrics and Gynaecology, Medicine (Division of Endocrinology), Educational Psychology, and Family Studies, University of Alberta.
Reprint requests: Dr. David C. Cumming, 1D1, Walter Mackenzie Centre, University of Alberta, Edmonton, Alberta, Canada T6G 2R7.
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Myths, popular beliefs, and attitudes toward menarche and menstruation

Menarche has little intrinsic endocrine significance inasmuch as it is neither the beginning nor the end of reproductive maturation, but it does symbolize a stage of maturity characterized by preparedness of the young woman's body for increased sexual responsiveness and a clearer sex role identification, and opens up consideration of possible future roles, for example, sexual partner and mother. One might expect that menarche would be positively received by young women as it provides an unequivocal indication that development is unfolding as it should, but both positive and negative responses have been described. On the positive side, menarche leads to clearer expression of a girl's body image and a greater acceptance of womanhood.[1,2] More negative responses such as increased shame, fear, anxiety, and depression have also been noted. The manner

in which a girl learns about menstruation and its associated changes may have an impact on her response to the event and her acceptance of herself as a woman.[3]

The menstruating woman of biblical times was considered unclean and profane,[4] a belief that persists. Until quite recently, men often regarded her as "disgusting."[4] Young girls soon learn that menstruation is not to be discussed in public.[5] Despite greater physiological knowledge, the attitude toward menstruation as the "curse" is overwhelmingly negative and a source of embarassment to be concealed. Even today it is often assumed that physicians will not conduct a pelvic examination during menstruation.

Several studies document the limited knowledge young women have about menstruation. The composition and normal volume of menstrual flow was not known and a quarter believed that the flow rids the body of wastes.[4] Whereas 74% of the respondents knew that ovulation referred to ovum release, 61% had no knowledge of when ovulation actually occurred in the menstrual cycle and 56% did not know when pregnancy was likely to occur, findings with implications for both fertility and contraception. Many young people still believe that pregnancy cannot occur at first intercourse.[6]

Old wives' tales persist. Many young women believe that they should avoid cold, heat, bathing, swimming, and exercise during menstruation.[4,6] During the 1960s there was considerable debate in education literature over the advantages and disadvantages of excusing girls from physical education classes during menstruation. A tradition developed that menstruation was globally normal and should not interfere with any physical education or other program. A long-term exercise program does help to reduce perimenstrual symptoms and physical activity during menstruation need not be avoided by the majority. However, young women during an episode of painful menstrual cramping could end up feeling that their experience is being negated by inappropriately vigorous encouragement to participate.

There are inaccurate beliefs about affective state and cognitive processing during menstruation. Of the 1034 respondents in the North American study reported by McNab,[6] 87% believed that women are particularly emotional while menstruating. A third of respondents believed that menstruation affects a woman's ability to think. In another study, 75% of male college students and a surprising 32% of female college students believed the same thing.[7] Of 1034 young men and women over the age of 14 years, 25% believed tht women could not function normally at work in the premenstruum.[6] Most women experience no significant cognitive or affective changes premenstrually and can perform any of their life functions in a normal fashion during the premenstruum.[8,9] Young men and women both attribute negative emotions to biologic events (e.g., those of the menstrual cycle) and positive feelings to life events.[10] Negative expectations about menstruation develop even before puberty.[11]

However, not all findings are negative. Young women from higher socioeconomic backgrounds are more likely to possess more accurate knowledge about menstruation.[4] Young college students accepted menstruation rather routinely,[12] which suggests that appropriate education could eliminate many of the negative expectations of menstruation and perhaps many dysfunctional symptoms.

It is important that physicians be aware that young women may have a limited understanding of the biologic and physical changes that are occurring and be prepared to validate experiences and guide the educational process when this is appropriate. Physicians may also harbor negative attitudes and beliefs about menstruation and should take care not to add to the patient's impression.

Sources of information

Education about menstruation ideally should be just one topic in a comprehensive, lifelong process of sexuality education; it should be provided before puberty and build on a base of sexuality education provided by parents early in a child's life. Sexuality education should begin at home and continue as reproductive status changes over the life span. Before puberty, menstrual education is a natural entrée to informed discussion of sexual development and of the entire reproductive process including physical, social, and psychological aspects of coitus, conception, and birth. The actual situation, with respect to menstrual education as one part of sexuality education, differs from what is ideal. Even today many young women are not prepared for menarche. For others, education about the hygienic aspects of menstruation is the only aspect of sexuality education that they will receive.

Young girls receive information about menarche and menstruation from a wide variety of sources: parents, peers, teachers, medical personnel, commercial materials, television, and other media. These sources not only vary in quality of information provided but also in the value young girls give them as a potential information source. Whereas there is still controversy over sex education, North Americans overwhelmingly believe that both parents and schools should be involved in the sex education process.[13] However, it appears that it is one thing to believe that home and school should be involved and another to accomplish it. Both young persons and parents agree that many fewer parents provide sex education than those who believe that they should provide it.[13-16] Peers have a strong influence on adolescents' sexuality education.[13,16] Schools are much

less influential because comprehensive programs are conspicuously absent in many communities.

Concern may be expressed not only about the effectiveness of the sources of information but also the affective tone with which it is transmitted, particularly with respect to perpetuating the negative views of menstruation. Parents often have difficulty teaching their children about sexual matters such as menstruation. It is not uncommon for parents to feel embarrassed as well as unprepared to carry out this task effectively. Parent's lack of accurate information will perpetuate myths that they acquired.

Physicians generally have had no instruction on how to teach reproductive information to others who are not medically trained. Most learn from doing and may well not be as effective as they would wish to be. We usually are little involved in the formal education process as it relates to reproduction and menstruation. Despite the time constraints and difficulty of communication with teenage and preteenage patients, the physician who deals with young women should facilitate and be prepared to be involved.

Two key objectives should guide physicians who respond to young women seeking information about menarche and menstruation: (1) to provide accurate information about the nature of bodily functions and their relationship to other aspects of reproduction (sexual responsiveness, reproduction) and (2) to facilitate the development of positive attitudes in young women about their own sexuality. Closed communication leading to the persistence of myths and mistaken impressions associated with menstruation can prevent the achievement of the first objective. Encouragement of young women to see menstruation as a monthly physical and emotional crisis rather than a positive, normal event may interfere with the achievement of the second objective.

School programs have suffered from similar problems. The mandate for schools to teach about sexuality is far from clear. Like physicians, most teachers have not been formally prepared to teach sexuality and therefore must depend on in-service education for their personal preparation. When school education programs have been implemented, there has been a high dependence on the education materials provided by commercial paper product companies that sell sanitary products. Films are the most widely used teaching aid: an estimated 93 million women have seen the animated film *The Story of Menstruation* since 1973. Booklets produced by paper companies are the second most widely used resource.

Because similar materials may be available through physicians, we should be aware of the kind of information provided by the commercial sources, whose content is very much influenced by analyses of the intended consumer markets. The information is often ritualistic and rule bound, for example, specifying how a girl should feel rather than facilitating her self exploration. The materials are not adequate as a comprehensive educational resource because of vagueness in purpose and language; the cold, clinical presentation of body diagrams do not accurately present the systemic character of the reproductive system; there is denial of unpleasant body feelings or sensations and emphasis on hygiene used so that menstruation can be concealed. Whereas they present a systematic idea of how to manage this natural body function in a sanitary, hygienic and "dainty" manner, they do not facilitate the exploration of the range of feelings (e.g., sensual, fearful, anxious, happy) that may accompany the arrival of puberty. The emphasis on independence and giving the facts and information on products has implications as well for limiting parent-daughter communication. The nature of the materials written for mothers of normal girls discouraged ongoing open communication between parent and child about the girl's affective responses to menstruation or other sexuality issues. In contrast, the materials written for mothers of retarded girls not only gave more concrete examples about the event but also encouraged ongoing and open communication. It may be concluded that an unrealistic, adaptive rather than realistic and dynamic developmental model is presented to the young girl to incorporate this change into her life.

Three patient education pamphlets on reproductive matters are published by the American College of Obstetricians and Gynecologists (600 Maryland Avenue, SW, Suite 300 East, Washington, DC), two directed to teenagers (*Growing up* and *Being a teenager: you and your sexuality*) and one for parents *(Teaching your children about sexuality)*. The pamphlet for parents provides understanding of the difficulties that parents face in teaching their children but the tone is generally too philosophical with few concrete suggestions about breaking the ice. *Growing up* focuses on hygienic management with too little comment on the concrete aspects of the period and the often mixed personal feelings. *Growing up* would benefit from the approach of *Being a teenager: you and your sexuality* in which the tone is positive and covers sexuality, not mechanics. However, it does not deal with menstruation.

Newer books about the menstrual cycle have provided a much broader focus on sexuality. These include *Menstruation: Just plain talk* by A. Nourse,[17] *Every girl* by H. Doan and J. Morse,[18] *Period: A book about menstruation* by Gardner-Loulan, Lopez, and Quackenbush,[19] and *What's happening to me?* by P. Mayle.[20] They were written with the objectives of helping the girl to understand the body changes associated with menstruation and how this process might be managed but, more impor-

tant, they focus on development of a good body and self-image and a feeling of satisfaction with being a woman. The books do not play down the natural, within-gender variabilities of body functioning with respect to menstruation and they deal realistically and accurately with the problems that women have suggested they experience with menstruation.

Informal sources such as prime-time television programs and advertisements may convey attitudes about sexuality that we may not wish young people to assume.[21] Certainly, this is relevant to menstrual education inasmuch as advertisements for menstrual products on television and in the media in general tend to convey the attitude that managing this body event is a monthly crisis of hygiene.

It is in our interests as physicians to become involved in the debate over sexuality education and to review standards of the sources of information. Information that after review is considered helpful can be recommended. It is clear from the attitudes of well-educated young women that an educational process can lead to healthier attitudes toward reproductive function and reduce many of the minor problems that are overtly or covertly troublesome. However, physicians should not see the task of sexuality education as merely information exchange or delegate the provision of information to others but be prepared to integrate other sources into their patient management. The provision of reading materials should be viewed as a complement to direct, open physician-patient communication rather than a substitute for it.

Future directions for menstrual education

The task for physicians and other sexuality educators with respect to menstrual education is not simple. First, a number of formal and informal partners are involved in the educational process who may provide conflicting information. Second, there are changes in educational processes (e.g., attitudes toward premenstrual symptoms, concepts of the relationship between a girl's experiences at puberty and subsequent identity formation, and information about the relationships between cognitive or affective development, and educational decisions such as the level of abstraction one should use or the timing and sources of information). Third, the success of any program of menstrual education is closely linked to a larger program or experience of sexuality education.

To chart new directions for menstrual education we need to address the components of the educational task and need the support and involvement of physicians in the community. The third component is the most important and incorporates the other two. Menstrual education should not be an isolated educational event. Thus a framework for sexuality education on the basis of lifelong developmental principles is needed. An understanding of young women's physical, emotional, and social development should provide the basis for identification of the needs for a comprehensive sexuality program and the placement of menstrual education within that developmental process. This same understanding will allow physicians and formal educators to recognize the different roles and impact of the various information sources about menstruation. The power of peers, parents, commercial companies, books, and other media as educational agents cannot be ignored. Formal educators in school or community contexts need to be clearer about devising methods to work cooperatively with these other informal educators. The changing knowledge base with respect to sexual functioning demands that educators be diligent in their personal updating. An informed stance allows all of us to evaluate education materials and to challenge current day mythology about sexual functioning.

The general and specialist reproductive physician must accept responsibility in the educational process. Numerous opportunities exist in the medical care setting to establish the rapport needed to answer sensitive questions and concerns that adolescents have about sexuality. Certain attitudes are necessary for the physician when caring for adolescents, particularly willingness to listen and being available to the adolescent when needed. In some cases this may mean adjusting patient practices so that some after-school appointments are reserved for adolescent patients and making efforts to make them feel comfortable when making an appointment or being received by the office personnel. Assumption of care of an adolescent implies that the adolescent, rather than the parent, is recognized as the patient and is treated with the same respect and courtesy as other patients. Adolescents desire a relationship with their physicians on the basis of honesty, respect, expediency, simplicity of education offered, involvement in the therapeutic modality, and encouragement of responsibility for oneself.[22] It is important early in the development of the physician-patient relationship to emphasize the confidentiality of the communication that will occur so that trust may develop. In this case both adolescents and their parents need to understand this principle. Adolescents must feel secure that they are speaking in confidence and may also expect to receive information within a health rather than moral paradigm. Physicians are viewed as respected authorities. On this basis as well as on the basis of a physical examination, the physician can reassure the adolescent of the normality of pubertal developments.

Physicians who desire involvement in the sexuality education of their adolescent patients need to begin to develop effective relationships with them before puberty. Such relationships are characterized by open

communication of feelings and physical concerns. Physicians need to be able to identify the crucial issues of development for these young patients and to initiate the discussion by posing questions and expecting to answer them. Adolescent patients often need "permission" to ask questions about sexuality. Physicians can establish an askable atmosphere by initiating this process. "How are you feeling about growing 6 inches last year?" "How are you feeling about not getting your period at the same age as your best friend? Some girls worry about this but I want you to know that every woman has her own timetable. Don't be afraid to give me or my nurse a call if you have questions about menstruation or sexuality." Information provided to young women should not only be factually accurate but also applied to the young woman's personal concerns. We should recognize that factual information is not all that young women need to know—reproduction is a systemic process and we must address the psychologic impact of the changes as well as the physical. This provides a greater sense of being prepared for puberty.[23]

A well-chosen resource library on puberty, sexually transmitted diseases, and sexual decision making may become a popular extra service for teens and their parents. Booklets should be available in the office but must be previewed before being placed for patients. A reading list could provide an alternative if books or pamphlets cannot be made available. Other methods to provide information and establish an approachable environment are to offer some of these books on a lending basis and schedule a repeat visit for the teen to ask questions or hold special group sessions for teens with the physician and the office nurse. The physician and the office nurse can operate well as an education team within the office structure. Above all physicians must be aware of normal reproductive physiology and be prepared to help young women to develop positive attitudes and an accurate knowledge base of their own reproductive function. Menstrual education needs to move away from the focus on hygienic management to that of healthy sexuality and acceptance of self.

Any sexuality educator has a sensitive task. Calderwood[24] suggests that to prepare for the task effectively there are four essential steps: (1) self-evaluation of one's own attitudes, beliefs, and knowledge of sexuality; (2) unlearning of the debris of myths and misunderstandings that may have been part of the educator's experience; (3) relearning, and finally (4) practice. It is not unusual for physicians to feel uncomfortable handling this task at first. Practice and participation in experiential workshops that address sexuality education or communication can assist the physicians in gaining the confidence that makes the sexuality education process an integral part of adolescent health care. Such a shift to a comprehensive view of menstrual and sex education offers a much greater potential for girls to validate their experience and to integrate this into their sense of self.

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Inhibition of pulmonary surfactant function by meconium

David Moses, MD,[a] Bruce A. Holm, PhD,[a, b, c] Patricia Spitale, MD,[b] Mingyao Liu, MD,[a] and Goran Enhorning, MD[a]

Buffalo, New York

The pathophysiology of meconium aspiration is marked by lung hyperinflation because of airway obstruction, which is often followed by an acute pneumonitis with classic lung injury characteristics. Surfactant dysfunction may contribute to this latter pulmonary pathophysiology. We sought to determine to what extent meconium itself might contribute to a functional surfactant deficiency. Specimens of newborn infants' first meconium were collected and pooled. Serial dilutions of the meconium were then added to various concentrations of calf lung surfactant extract, a mixture with the surface properties of natural surfactant that is used clinically to treat neonatal respiratory distress syndrome, and the dynamic surface activity of these mixtures was studied with a pulsating bubble surfactometer. At surfactant concentrations of ≤1.5 mg/ml, even 6500-fold dilutions of meconium-inhibited surface tension lowering ability (10 ± 2 mN/m vs 1 ± 0.1 mN/m for controls, $p < 0.05$). Moreover, this inhibitory activity resided in both the chloroform-soluble and the aqueous phases of meconium and appeared to be additive in nature. However, at sufficiently high concentrations of surfactant, even large amounts of meconium were unable to affect surface tension lowering properties. Thus meconium inhibits surfactant function in a manner that is dependent on the surfactant concentration, suggesting the possible utility of exogenous surfactant therapy in some cases of meconium aspiration. (Am J Obstet Gynecol 1991;164:477-81.)

Key words: Surfactant inhibition, meconium

Meconium is a mixture of bile salts, cell debris, mucopolysaccharides, cholesterol, and enzymes from the fetal intestinal mucosa and biliary tract.[1] Meconium staining of amniotic fluid occurs in 10% to 18% of all deliveries, and 55% of these infants have meconium in the trachea.[2, 3] Meconium aspiration, occurring in 1% to 3% of all term and postterm births, has been associated with severe neonatal respiratory distress characterized by hypoxemia, intrapulmonary shunting, and decreased lung compliance.[2]

Although some of the pathophysiology of meconium aspiration syndrome is clearly related to mechanical obstructions in the airways, previous studies have shown that meconium-induced pulmonary dysfunction may also be related to chemical effects on the lung.[4] These findings have generated comparisons of this component of meconium aspiration syndrome to other forms of disturbed lung function, such as the adult respiratory distress syndrome (RDS).[5]

Adult RDS may lead to acute respiratory failure in both adults and children after a variety of pulmonary or systemic insults, including sepsis, shock, liquid aspiration, pneumonia, oxidant injury, trauma, and many others.[5] Mortality rates for adult RDS have been reported to be as high as 50% to 70%.[5] Recent evidence from this laboratory and others indicates that pulmonary surfactant dysfunction may play a significant role in the development and pathophysiology of adult RDS.[6] The observed surfactant abnormalities in adult RDS appear to be related to the inhibition of surfactant biophysical activity by plasma proteins and other agents that enter the alveolar space during lung injury.[7-11] Moreover, this biophysical inactivation can be avoided by sufficiently increasing the surfactant concentration, suggesting the potential efficacy of exogenous surfactant therapy in adult RDS-type lung injuries.[6-11]

The studies presented here were designed to determine the effects of meconium and its components on

From the Departments of Gynecology-Obstetrics,[a] Pediatrics,[b] and Pharmacology and Therapeutics,[c] State University of New York at Buffalo.
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Reprint requests: Bruce A. Holm, MD, Perinatal Center, Children's Hospital of Buffalo, 219 Bryant St., Buffalo, NY 14222.
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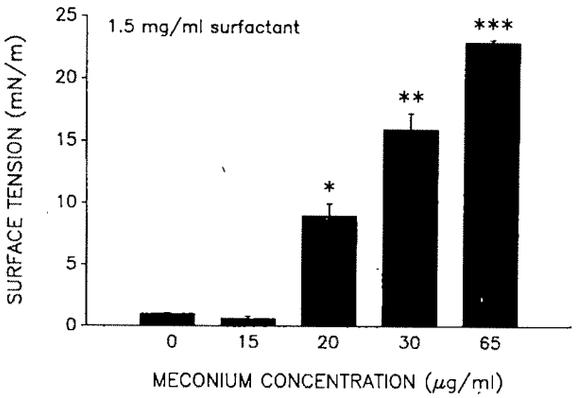


Fig. 1. Effects of meconium on 1.5 mg/ml surfactant. Various concentrations of meconium were added to 1.5 mg/ml surfactant. Results represent mean surface tension at minimum bubble size after 5 minutes of dynamic cycling on oscillating bubble. ($n = 8$ for each case). *Asterisk*, $p < 0.05$; *double asterisk*, $p < 0.005$; *triple asterisk*, $p < 0.0005$.

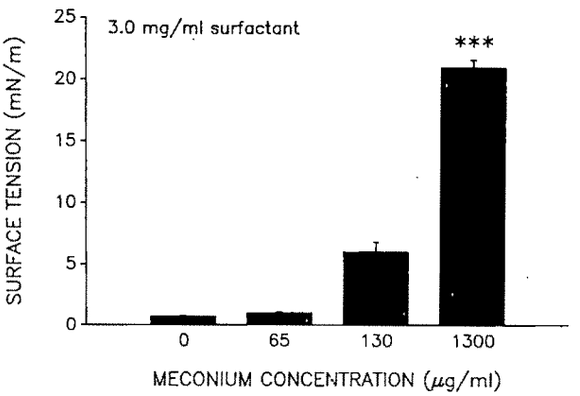


Fig. 2. Effects of meconium on 3.0 mg/ml surfactant. Results represent mean surface tensions at minimum bubble size after 5 minutes of cycling ($n = 8$ for each case). *Asterisk*, $p < 0.05$; *double asterisk*, $p < 0.005$; *triple asterisk*, $p < 0.0005$.

the biophysical activity of pulmonary surfactant. The experiments examined dilutions of meconium or meconium components that were not capable of forming obstructive masses, and their ability to cause surfactant dysfunction, as measured on an oscillating bubble surfactometer.

Material and methods

Preparation of lung surfactant. Natural lung surfactant was obtained by bronchoalveolar lavage of lungs from freshly killed calves as previously described.[12] Briefly, lungs were lavaged three times with 1 L of 0.15 mol/L sodium chloride infused and withdrawn intermittently with a vacuum line. The lavage fluid was centrifuged at 300 *g* for 6 minutes to remove cells and debris, and the supernatant was then spun at 12,500 *g* to pellet phospholipids and associated proteins. This material, subsequently resuspended in 0.15 mol/L sodium chloride, is referred to as natural lung surfactant and is 85% phospholipid and 10% protein.[12]

Preparation of calf lung surfactant extract. Calf lung surfactant extract was prepared from natural lung surfactant by extraction with chloroform and methanol as previously described.[8, 13] The calf lung surfactant extract mixture was >95% phospholipid with a class distribution identical to that of lung surfactant. However, the calf lung surfactant extract protein content was reduced to approximately 2%, representing the low molecular weight, hydrophobic surfactant apoproteins that are required for biophysical activity.[12] For biophysical studies calf lung surfactant extract was dried from chloroform under nitrogen and dispersed in 0.15 mol/L sodium chloride by mechanical mixing. This surfactant preparation has been used extensively in the clinical treatment of neonatal RDS.[13, 14]

Meconium preparation. Meconium samples were collected from 11 healthy infants in the nursery. The meconium was then pooled and blended in a minimal amount of normal saline solution to attain a stock meconium preparation at 130 mg dry weight per milliliter. Aliquots of the meconium pool were stored frozen at −20° C until use. Working meconium solutions were diluted appropriately with normal saline solution.

Meconium extractions. For some experiments meconium was extracted with chloroform and methanol by the method of Bligh and Dyer[15] to separate the water-soluble and chloroform-soluble elements of meconium. Each phase was taken to dryness by nitrogen and heat evaporation and subsequently resuspended at the appropriate concentration in 0.15 mol/L sodium chloride. Aliquots of these materials were stored frozen until use.

Phospholipid analyses. Phospholipid phosphorus analyses were determined by the method of Chen et al.[16] Composition analyses used thin-layer chromatographic techniques with the solvent system of Touchstone et al.[17]

Protein analyses. Protein determinations were done by a sodium dodecyl sulfate modification of the method of Lowry et al.,[18] which allows protein measurements in the presence of high concentrations of lipid.

Surface tension measurement. Adsorption and dynamic surface tension measurements were conducted at 37° C and 100% humidity on a pulsating bubble surfactometer (Electronetics, Amherst, N.Y.) as designed by Enhorning.[19] An air bubble was formed in 25 μl of sample and then pulsated, which compressed the surface area of the bubble by 50%, at a rate of 20 cycles per minute. The pressure difference across the

bubble surface was recorded continuously, and the surface tension values were calculated from the law of Young and Laplace as it applies to spherical surfaces:

$$P = 2\gamma/R$$

where P is the pressure difference across the bubble surface, γ is the surface tension, and R is the bubble radius.

Statistical analyses. Statistical analyses were performed with the Minitab computer software package (Minitab, Inc., State College, Pa.). Analysis of variance was performed on the various mixtures at each surfactant concentration. The unpaired Student *t* test was then performed and compared with control values for those mixtures determined significant by analysis of variance. Statistical significance was assigned for *p* values ≤ 0.05.

Results

Figs. 1 to 3 show the effects of meconium on the dynamic surface activity of pulmonary surfactant as measured on an oscillating bubble surfactometer. The graphs depict the surface tensions at minimal bubble size for various surfactant-meconium mixtures after bubble expansion and 5 minutes of pulsation.

The results in Fig. 1 indicate that at a surfactant concentration of 1.5 mg/ml, as little as 20 μg/ml meconium is capable of causing a significant increase in the minimum surface tension. This meconium concentration, which represents a 6500-fold dilution of the newborn meconium pool, prevented the surfactant mixture from reaching a surface tension below 11 mN/m, a value that is considered to be incompatible with normal physiologic function. Maximal inhibition of surfactant function occurred with 65 μg/ml meconium.

The data in Fig. 2 indicate that pulmonary surfactant at a concentration of 3 mg/ml is also inhibited by meconium, although higher meconium concentrations are required to generate the same degree of inhibition. At this higher surfactant concentration, inhibition of surface activity is first seen at 130 μg/ml meconium, representing a 1000-fold dilution of the natural meconium pool. However, maximum inhibition of surfactant function required 1300 μg/ml meconium. Although this still represents a 100-fold dilution of the original meconium material, the amount of meconium required to inhibit 3 mg/ml surfactant was much higher than one would predict on the basis of a simple stoichiometric interaction between surfactant and meconium.

More important, the results in Fig. 3 indicate that at a surfactant concentration of 6 mg/ml, even the addition of 25 mg/ml meconium, which is only a five-fold dilution of the original pool, was unable to cause significant inhibition of surfactant function. These results indicate that surfactant inhibition by meconium can be overcome at sufficiently high surfactant concentrations.

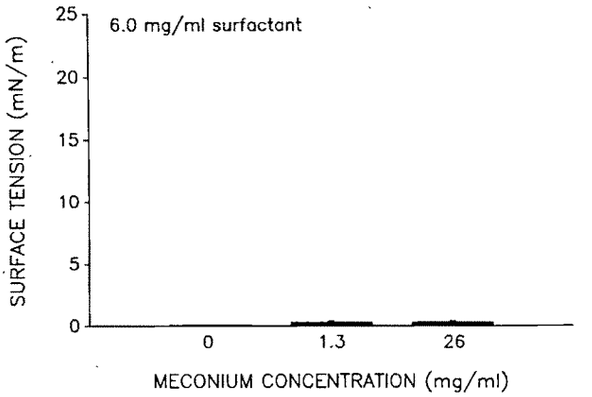


Fig. 3. Effects of meconium on 6.0 mg/ml surfactant. Results represent mean surface tensions at minimum bubble size after 5 minutes of cycling. See text for details.

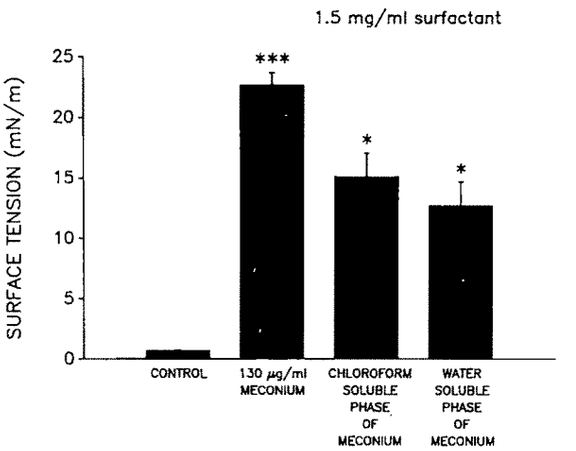


Fig. 4. Effects of meconium components on 1.5 mg/ml surfactant. Total of 130 μg/ml meconium or the associated chloroform-soluble and water-soluble phases were added to 1.5 mg/ml surfactant. See text for details. *Asterisk*, $p < 0.05$; *double asterisk*, $p < 0.005$; *triple asterisk*, $p < 0.0005$.

In an effort to determine the meconium components that are responsible for the surfactant inhibition, further studies were done in which various concentrations of meconium were separated into water-soluble and chloroform-soluble fractions by chloroform-methanol extraction. These components were then added to surfactant suspensions to determine effects on dynamic surface activity as shown in Fig. 4. The data indicate that significant surfactant inhibitory activity resides in both water-soluble and chloroform-soluble phases of meconium, and that the effects after 5 minutes of dynamic cycling appear to be roughly additive.

Comment

The pathophysiology of meconium aspiration syndrome is highly complex and is characterized by hypoxemia, intrapulmonary and extrapulmonary shunting, airway obstruction, and decreased lung compliance.[2,3] Therapy for meconium aspiration has included application of positive-end expiratory pressure, vasodilating agents, and extracorporeal membrane oxygenation.[3,20] The clinical course after meconium aspiration may be as variable as the suggested therapies.

Although many studies concentrate on the airway obstruction and "ball-valve" effects observed in meconium aspiration syndrome, several recent studies have focused on the chemical pneumonitis caused by meconium, suggesting that even small amounts of meconium may be able to produce lung mechanical abnormalities in the absence of mechanical obstructions.[4] The results presented here clearly show that meconium causes significant inhibition of the surface tension lowering properties of pulmonary surfactant. Moreover, these in vitro studies indicate that at lower surfactant concentrations, extremely dilute solutions of meconium are capable of causing a dramatic increase in the minimum surface tension to levels that are incompatible with normal physiologic function. However, at sufficiently high concentrations of pulmonary surfactant, even relatively concentrated meconium preparations were unable to significantly affect the ability of surfactant to lower surface tension under dynamic compression.

These findings are in agreement with previous studies[7-11] that showed that pulmonary surfactant activity can be inhibited by various agents, such as plasma proteins that enter the alveolar space during lung injury. These previous studies also showed that the inhibition was dependent on the surfactant concentration, such that above a threshold surfactant level even exceedingly large amounts of inhibitory proteins were without effect.[9] However, it is clear from the results presented here that the meconium concentrations needed to achieve an inhibitory effect were much lower than those required for plasma proteins and blood cell components.[7,8] Similarly, the necessary surfactant threshold concentration above which inhibition was no longer observed was much higher for meconium and its components than it was for plasma proteins. Thus it appears that meconium may be even more inhibitory of surfactant function than other agents found in the alveoli during different forms of lung injury.

This finding may be due in part to the complex composition of meconium. All individual components of meconium have not been completely defined, but it is known that meconium contains both chloroform-soluble (hydrophobic) components, such as cholesterol, free fatty acids, and glycolipids, and water-soluble (hydrophilic) components, such as mucopolysaccharides and proteolytic enzymes.[1] Our data indicate that both the water-soluble and chloroform-soluble components of meconium are capable of causing significant inhibition of surfactant activity. However, the exact determination of which constituents of meconium are responsible for the surfactant inhibition requires further study.

Similarly, further studies are needed to determine the mechanism or mechanisms by which meconium and its components inhibit surfactant function. On the basis of previous studies by Holm et al.[9] examining the mechanisms for plasma protein-induced inhibition of pulmonary surfactant activity, one might speculate that meconium may inhibit surfactant function by competing with surfactant molecules for space at the air-liquid interface during adsorption or intercalating into the surfactant film during dynamic compression.

The fact that meconium-induced inhibition of surfactant function is dependent on the surfactant concentration has at least two points of practical interest. First, it suggests that localized areas of the lung with a partial surfactant deficiency may be more susceptible to the detrimental effects of small amounts of meconium. Second, the results indicate that meconium-induced pathophysiology related to surfactant inhibition phenomena may benefit from exogenous surfactant replacement therapy. Indeed, previous work by this laboratory[6] showed that the use of surfactant therapy to treat lung injuries with components of surfactant inhibition by proteins resulted in significant amelioration of the associated pathophysiology.

In summary, the studies presented here show that very dilute preparations of meconium are capable of causing significant inhibition of the surface tension lowering properties of pulmonary surfactant, and do so in a manner that is dependent on the surfactant concentration. These results suggest that amnioinfusion with saline solution to reduce the incidence of cord compression and meticulous upper airway suctioning of infants with meconium-stained fluid may be very important. The results also allow us to speculate that exogenous surfactant therapy may prove useful for the treatment of some aspects of meconium aspiration syndrome. Clearly, further studies on the role of surfactant system alterations in meconium aspiration are warranted.

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The effects of iodoprophylaxis on thyroid size during pregnancy

Rossella Romano, MD,[a,*] Emmanuele A. Jannini, MD,[b,*] Mario Pepe, MD,[b] Alessandro Grimaldi, MD,[b] Michele Olivieri, MD,[b] Patrizia Spennati, MD,[b] Francesco Cappa, MD,[c] and Massimino D'Armiento, MD[b]

L'Aquila, Italy

Thyroid gland size was ultrasonographically determined in 35 pregnant women who live in an area with moderate iodine deficiency. Iodide salt was administered to group A ($n = 17$), whereas group B ($n = 18$) was used as a control. Each group was tested for thyroid-stimulating hormone serum levels, iodine excretion, and thyroid volume. In both groups thyroid-stimulating hormone levels were similar and did not change throughout pregnancy. The iodine excretion at the third trimester in the treated group was significantly ($p < 0.01$) higher than that of group B (100.0 ± 39.0 versus 50.0 ± 37.0 μg iodine per 24 hours, respectively). Initially, thyroid volume did not differ between the two groups. At the end of pregnancy, no difference was found in thyroid size in group A, whereas in the untreated women it increased significantly ($p < 0.0001$) with a mean increase of 1.6 ± 0.6 ml (16.2% ± 6.0%). These results show that the increased thyroid size in the control group was mainly a result of relative iodine deficiency and that iodoprophylaxis should be warranted even in areas with moderate iodine deficiency to prevent the increase in thyroid size and, probably, to avoid the risk of maternal and fetal hypothyroidism. (Am J Obstet Gynecol 1991;164:482-5.)

Key words: Iodine deficiency, iodoprophylaxis, goiter, ultrasonography

The relationship between pregnancy and the thyroid gland has long been recognized and remains a matter of controversy.[1-3] The enlargement of the thyroid gland was once thought to be a characteristic feature of pregnancy and in antiquity a neck measurement was used as a pregnancy test.[4] The goitrogenic effect of pregnancy is partially justified by the effect of estrogen on the production of thyroxine-binding globulin, by the thyroid-stimulating hormone (TSH) activity of human chorionic gonadotropin, and by the effect of thyroid-stimulating immunoglobulins.[5-7] However, it is suggested that the main causative factor is the relative iodine deficiency that occurs during pregnancy as a result of the increase in renal iodine clearance.[8] In fact, epidemiologic studies that report that goiter commonly occurs in pregnancy often derive from investigations conducted in regions where iodine deficiency is common.[9, 10]

The aims of this study are to investigate the thyroid size during pregnancy by use of an accurate ultrasonographic technique and to show the role of iodoprophylaxis in the prevention of an increase in thyroid volume in a group of pregnant women who live in an area with moderate iodine deficiency.

From the Departments of Internal Medicine,[a] Experimental Medicine,[b] and Surgery,[c] University of L'Aquila.

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Reprint requests: Massimino D'Armiento, MD, Division of Endocrinology, Department of Experimental Medicine, University of L'Aquila, Collemaggio, L'Aquila, Italy 67100.

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Subjects and methods

Subjects. There was a random selection of pregnant women for the study group from the Obstetrics and Gynecology Department of the University of L'Aquila, L'Aquila, Italy. Only pregnant women who had a normal pregnancy and no personal history of thyroid diseases or other disease abnormalities at the ultrasonographic examination were included. None of the women was pregnant during the previous year and none of the subjects was taking any drugs that could have affected the thyroid hormone metabolism. Pregnant women who had a mean age of 27.1 ± 3.8 years and a mean body weight of 61.6 ± 4.9 kg at the first examination during the first trimester were randomly allotted to group A ($n = 17$). Pregnant women in group B ($n = 18$) had a mean age of 27.1 ± 2.9 years and a mean body weight of 60.9 ± 4.0 kg. All the subjects were born in and live in L'Aquila. The regular smokers (5 to 20 cigarettes per day) were randomly allotted to the two groups. Immediately after the first examination, iodide salt containing 20 mg iodide per kilogram, equivalent to a daily intake of about 120 to 180 μg iodine, was prescribed to all the women in group A.

Each trimester all the pregnant women in both

Table I. Age, body weight, thyroid volume, and iodine excretion at the first evaluation in both groups (mean ± SD)

	No.	Age (yr)		Body weight (kg)	Thyroid volume (ml)	Iodine excretion (μg/24 hour)
		Mean	Range			
Group A	17	27.1	21–35	61.6 ± 4.9	9.8 ± 1.9	37.0 ± 36.0
Group B	18	27.1	23–33	60.9 ± 4.0	10.1 ± 2.3	30.5 ± 42.0

Table II. Iodine excretion (μg/24 hours) in both groups at each trimester (mean ± SD)

	Trimester		
	1	2	3
Group A	37.0 ± 36.0	154.0 ± 59.0*	100.0 ± 39.0†
Group B	30.5 ± 42.0	55.0 ± 35.0	50.0 ± 37.0

*$p < 0.0001$.
†$p < 0.01$ vs first trimester.

groups were subjected to three ultrasonographic evaluations of thyroid volume and to measurement of body weight.

During each examination 24-hour urine samples were taken to determine the iodine urinary excretion by the Riley and Gochman method[11] with a Technicon autoanalyzer. Serum samples were stored at −20° C to test TSH values by ultrasensitive assay (RIAgnost, Behring, Italy). Each patient's samples were analyzed in duplicate in a single radioimmunoassay run. The range of normal values is 0.25 to 4.1 μU/ml and the intraassay coefficient of variation was 3.0%.

Methods. Thyroid volume was measured in all the subjects by ultrasonography with a real-time ultrasound sector scanner (SIM 4000, Esaote Biomedica, Florence, Italy) with a 7.5 MHz transducer. The same investigator was used to obtain ultrasonographic measurements (R.R.) with the subjects in the supine position with hyperextension of the neck. Subsequent measurements were taken not knowing the initial dimensions of the thyroid and to which group the women belonged. The volume of each lobe was determined by use of the formula for the volume of an ovoid: $\pi/6$ (D × W × L),[12] where D is the anterior-posterior dimension of each lobe that was obtained from film images of transverse and sagittal ultrasonographic scans, W is the lateral dimension that was obtained from the film images of the transverse scan, and L is the longitudinal dimension calculated from the film images of the sagittal scan. The sum of the two lobe volumes equals the total thyroid volume. The degree to which repeated computations were reproducible (i.e., the precision of the ultrasonographic method) was estimated by determination of the thyroid volumes in 20 nonpregnant healthy women on day 2 of their menstrual cycles.[13] On the basis of this estimation, the coefficient of variation was 4.8% ± 0.3%.

Informed consent was obtained from each subject.

Values were expressed as mean ± SD. Comparisons were made by use of the Student t test and the linear correlation coefficient between two variables. Significance was defined as $p < 0.05$. Pearson's linear correlation coefficient was used to analyze the association between two variables.

Results

The variables that could influence goiter frequency during pregnancy (i.e., parity, age, body weight, and smoking habits) were similar for both groups studied. At the first evaluation, no significant differences were found with regard to age, body weight, thyroid volume, and urinary iodine excretion (Table I). Similarly, the variation of weight was similar during pregnancy (15.0 ± 4.0 vs 14.3 ± 2.2 kg). In addition, no statistical correlation was found between the percentage of variation of thyroid volume and body weight or age in both groups ($p = 0.78$, $r = -7994$, and $p = 0.87$, $r = 0.046$, respectively).

During pregnancy, TSH levels of all the patients were within the normal range. For each trimester the TSH levels did not statistically differ between women of the same group and between the two study groups.

A significant increase in urinary iodine excretion was found at the second and third examination ($p < 0.0001$ and $p < 0.01$, respectively, Table II) only in group A, treated with iodide salt.

Thyroid volume did not change significantly throughout pregnancy in the group treated with iodide salt, whereas in the control group it increased significantly ($p < 0.0001$), with a mean increase of 1.6 ± 0.6

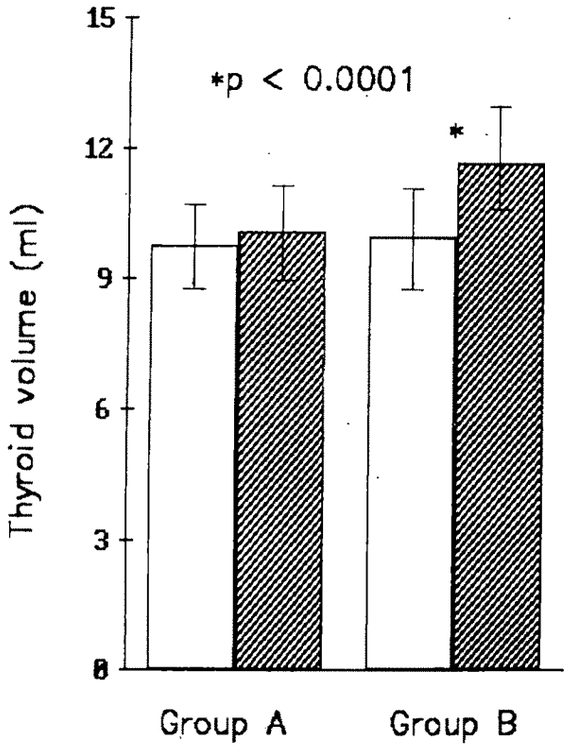


Fig. 1. Variation in thyroid volume (mean ± SD) in 17 women treated with iodide salt (group A) and in 18 untreated women (group B) between the first *(open bars)* and third *(hatched bars)* trimester. (*$p < 0.0001$ vs group A.)

ml (16.25% ± 6.29%) between the first and third trimester (Fig. 1).

Comment

The enlargement of the thyroid gland was once thought to be a characteristic feature of pregnancy.[14, 15] This belief can be questioned from recent epidemiologic observations that failed to show this correlation to geographic areas where iodine intake is adequate.[3] The results of this clinical study showed the need of an adequate dietary iodine intake to prevent the development of a gestational goiter. In addition, we directly showed that iodine deficiency is the main causative cofactor of thyroid enlargement during pregnancy.

In the Abruzzo region the prevalence of a goiter (groups 1B, 2, and 3 of the World Health Organization) in the 8- to 15-year-old population is 41%, mean urinary iodine excretion is 45 ± 12 μg/24 hours, and 98% of the drinking water sources have an iodine concentration <1.3 μg/L.[16] Of all the pregnant women who live in this area, which has a moderate iodine deficiency, only those who use iodide salt and show values of urinary iodine excretion ≥100 μg/24 hours at the third trimester did not have a significant increase in thyroid size. On the contrary, in the untreated group that had lower values of urinary iodine excretion, a significant increase in the mean thyroid volume was observed at the end of the study period. Because urinary iodine excretion accurately reflects dietary iodine intake, one can therefore infer that to prevent the enlargement of the thyroid gland during pregnancy, a minimum intake of 100 μg/day of iodine is required. The iodoprophylaxis with iodide salt containing 20 mg iodide per kilogram ensures an adequate daily iodine intake for the population that lives in this area. However, the recommended reduction in salt intake to prevent gestational hypertension may cause an insufficient iodine intake. In this case, salt with iodide concentration >20 mg/kg should be available.

Contrary to what has been reported by Hegedus,[17] but in agreement with others,[12] our results show that the increase in thyroid volume is unrelated to age and body weight variations. In addition, in agreement with a recent report,[18] TSH serum levels were normal in all the women and did not change during pregnancy. This also applies to the untreated women who showed a significant increase in thyroid volume. This may be explained by the experimental demonstration that alterations in thyroid blood flow are reciprocal to iodine intake levels and appear to be independent of the circulating THS levels. Furthermore, it has been shown that iodine can inhibit thyroid cell growth by a TSH-independent mechanism.[19] Therefore our results confirm that the increased renal iodine clearance is the major pathogenetic mechanism of thyroid gland enlargement during pregnancy.[8]

The urinary iodine excretion of the population studied during the first trimester (Table II) is low when compared with the values recommended by the World Health Organization (150 to 200 μg/24 hours). Because thyroid function abnormalities are frequent under the critical value of 30 μg/24 hours, our population is at risk of development of a syndrome now termed *iodine deficiency disorder*.[20] As far as the mother is concerned, it can cause miscarriages and stillbirths, whereas in the offspring it can cause congenital abnormalities, goiter, hypothyroidism, and impaired brain function.

In summary, in normal iodine intake conditions during pregnancy there is no change in the thyroid volume. This suggests that any enlarged thyroid, even in pregnancy, is diseased. To prevent iodine deficiency disorder, which is the major preventable cause of mental defects in human beings,[20] a supplementation of at least 100 μg/day of iodine during pregnancy should be guaranteed even in moderate iodine deficiency areas.

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Investigation of placental circulations by color Doppler ultrasonography

Eric Jauniaux, MD,[a, c] Davor Jurkovic, MD,[b] Stuart Campbell, MD,[a] Asim Kurjak, MD,[b] and Jean Hustin, MD, PhD[c]

London, England, Zagreb, Yugoslavia, and Loverval, Belgium

The placental circulations of 25 normal and five complicated pregnancies were studied by color Doppler ultrasonography. Flow velocity waveforms were obtained in all 30 pregnancies and could discriminate between fetal and maternal intraplacental blood flow. We believe that color Doppler ultrasonography will improve our understanding of the pathophysiology of various pregnancy disorders that alter the placental circulations and that color ultrasonography is useful for the prenatal differential diagnosis of intrauterine masses. (AM J OBSTET GYNECOL 1991;164:486-8.)

Key words: Placenta, color flow imaging, Doppler ultrasonography, prenatal diagnosis

Before the development of ultrasonographic techniques our knowledge of the anatomy and physiology of the maternal and fetal placental circulations in human beings had to be extrapolated mainly from data of in vitro perfusion experiments.[1] The advent of Doppler ultrasonography provided us with a new noninvasive method for the in vivo study of the placental circulations. Color Doppler ultrasonography made it possible to image and to investigate small vessels within fetal organs.[2]

The aim of this work is to evaluate the possible applications of color Doppler ultrasonograpny for the study of the morphology and the pathophysiology of the placental circulations.

Material and methods

Thirty singleton pregnancies between 18 and 42 weeks' gestation were studied by means of color Doppler imaging (Aloka Color Doppler SSD-680, Aloka Color Company, Tokyo, Japan).

The study group comprised 25 normal pregnancies, two pregnancies complicated by preeclampsia and intrauterine growth retardation, two pregnancies with hypoechoic tumor of the placental margin, and one pregnancy referred for detailed ultrasonographic examination because of a large heterogeneous intrauterine mass.

Conventional real-time imaging was performed in each pregnancy to identify the different placental structures. With the use of color imaging a prospective search was made for umbilical and chorionic vessels, uterine arteries, and uteroplacental and intraplacental vascular network. For each segment, resistance and pulsatility indexes were measured by spectral analysis of flow velocity waveforms obtained by pulsed Doppler ultrasonography. A nonparametric test (Kruskal-Wallis rank test) was used to determine whether differences in resistance increment between these segments were significant.

Results

Table I summarizes the mean values (± SEM) for resistance and pulsatility indexes obtained from the different segments of the placental circulations.

The waveforms obtained from the fetal side showed the same pattern with a significant decrease in flow resistance going proximal from the fetus into the substance of the placenta (Fig. 1, *A* and *B*).

Within the maternal circulation the resistance also decreased significantly toward the placental bed. A distinct blood flow pattern was found in the terminal segment of the uteroplacental circulation (Fig. 1, *D*). This flow pattern was identified at the level of the maternal plate and up to a few millimeters inside the uterus. It showed a lower resistance and turbulences as compared with the main uterine and radial arteries waveforms. A venous flow pattern (Fig. 1, *C*) was identified in placental sonolucent areas located in the center of the cotyledons or under the chorionic plate.

High-resistance and pulsatility indexes were found in all segments of the uteroplacental circulation in the two pregnancies complicated by preeclampsia. Higher indexes were also found in all segments of the fetal circulation and pathologic examination showed multiple placental infarcts in one of these pregnancies.

In one of the two pregnancies complicated by the presence of a "tumor" at the margin of the placenta,

From the Obstetric Ultrasound Unit,[a] Departments of Obstetrics and Gynaecology, King's College Hospital, King's College School of Medicine and Dentistry, the Ultrasonic Institute,[b] University of Zagreb, and the Histopathologic Institute of Loveral, Belgium.[c]
Received for publication June 27, 1990; revised September 5, 1990; accepted September 10, 1990.
Reprint requests: Eric Jauniaux, MD, Department of Obstetrics and Gynaecology, Academic Hospital Erasme, 808 Route de Lennik, Brussels, Belgium 1070.
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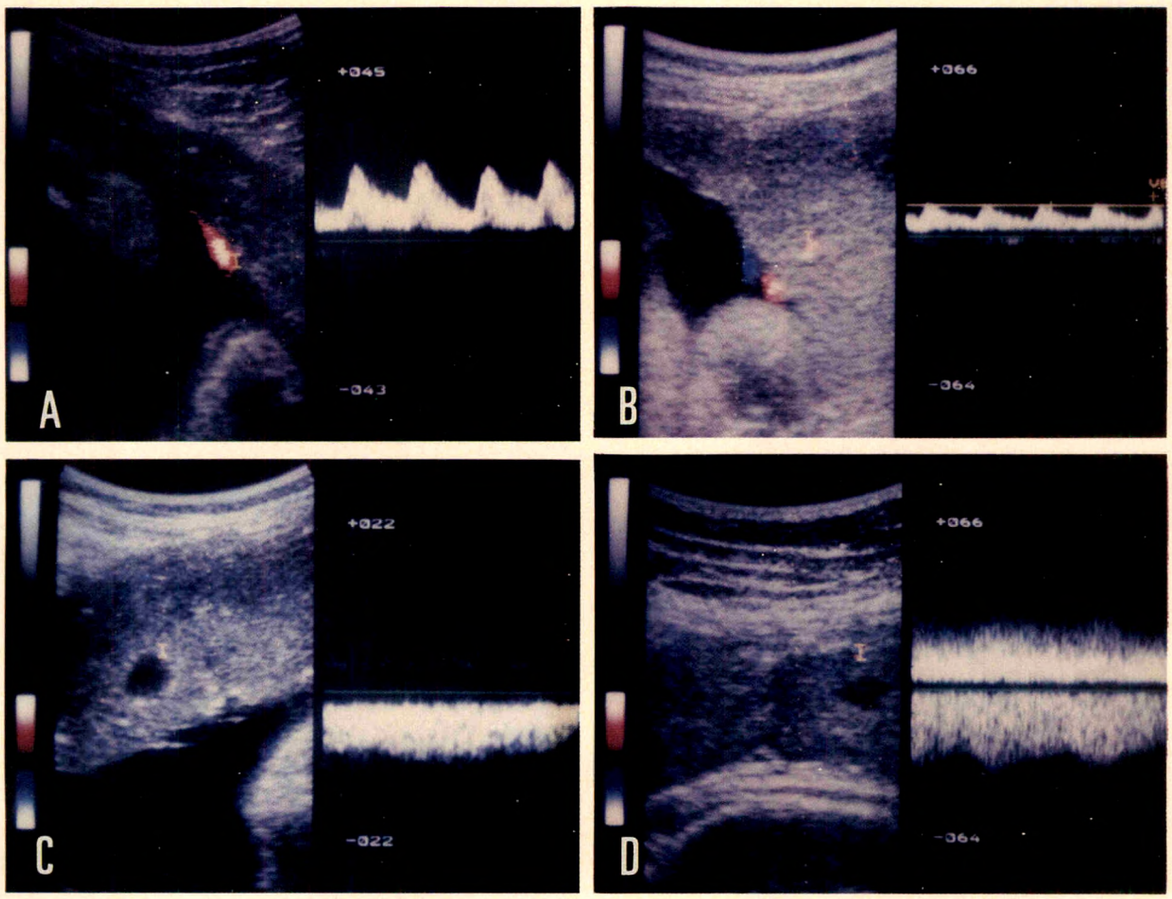


Fig. 1. Color flow mapping and flow velocity waveforms of different segments of fetal and maternal intraplacental circulations. **A**, Chorionic artery at 28 weeks' gestation; **B**, intraplacental arterioles at 32 weeks' gestation; **C**, venous flow pattern in periphery of a sonolucent area at 38 weeks' gestation; **D**, low-resistance turbulent flow at the level of the maternal plate, under a sonolucent area, at 40 weeks' gestation.

the waveforms obtained from the lesion were of maternal origin, suggesting a uterine myoma. In contrast, in the other pregnancy, waveforms with fetal characteristics were identified within the lesion. In the latter pregnany, pathologic examination confirmed the presence of a chorioangioma located at the edge of the placenta.

Color Doppler imaging did not show any blood flow within the abnormal intrauterine mass that was found to be associated with an anatomically normal fetus and a normal placenta. These features suggested molar tissue rather than a degenerating myoma or another uterine tumor where maternal signals would be expected. Pathologic investigations showed a twin pregnancy that combined a classic hydatidiform mole and a normal pregnancy.

Table I. Mean values (± SEM) for resistance and pulsatility indexes obtained from the placental circulations

	Resistance index	*Pulsatility index*
Fetal circulation		
Umbilical artery	0.662 ± 0.027	1.145 ± 0.050
Chorionic artery	0.564 ± 0.017*	0.848 ± 0.035*
Intraplacental arteriole	0.461 ± 0.014*	0.692 ± 0.040†
Maternal circulation		
Uterine artery	0.559 ± 0.014	0.916 ± 0.051
Radial artery	0.404 ± 0.010*	0.546 ± 0.020*
Spiral artery	0.326 ± 0.008*	0.422 ± 0.018*

*$p < 0.001$ significantly different increment from preceeding value (Kruskal-Wallis rank test).

†$p < 0.01$ significantly different increment from preceeding value (Kruskal-Wallis rank test).

Comment

Color Doppler ultrasonography is changing our ability to map the fetal and maternal components of the placental circulation. In this study, small branches of both uterine and umbilical cord arteries were clearly visualized with the color mode (Fig. 1). The two-dimensional display of these vessels allowed accurate placement of the pulsed-Doppler gate for spectral anal-

ysis. Identification of chorionic vessels, intraplacental fetal arterioles, terminal branches of the uteroplacental vessels, and intervillous blood flow could be easily performed in all pregnancies. A significant fall of resistance on both fetal and maternal sides was observed (i.e., from the umbilical artery to chorionic arteries and to intraplacental arterioles and from the uterine artery to the radial and spiral arteries; Table I).

The overall structure of placental vasculature as shown with color imaging and spectral analysis correlated well with the classical anatomic findings.[1] Normal placentas at term contain avillous cavities of various sizes and shapes located mainly in the center of the cotyledons. Perfusion studies in vitro showed that these cavities are a result of the spurt of maternal blood that comes from the spiral artery and pushes away the surrounding villous tissue.[1] Sonolucent spaces identified by real-time imaging contain turbulent flow on color imaging with a venous pattern on spectral analysis (Fig. 1, *C*). When the Doppler gate is moved from these spaces toward the basal plate of the placenta, waveforms with distinct maternal characteristics and assumed to derive from the spiral arteries can be seen (Fig. 1, *D*). Accurate identification of these flows appears promising in understanding the pathophysiology of placental vascular lesions such as thrombosis or infarcts and placental insufficiency.

Knowledge of the expected fetal or maternal waveform characteristics is also important if color Doppler ultrasonography is to have a role in differential diagnosis between placental and other intrauterine abnormalities. For instance, in cases of undetermined placental mass, demonstration of fetal waveforms strongly suggests chorioangioma, whereas maternal waveforms favor the diagnosis of a uterine myoma.

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Reduction of cardiovascular disease–related mortality among postmenopausal women who use hormones: Evidence from a national cohort

Pamela H. Wolf, DrPH,[a] Jennifer H. Madans, PhD,[b] Fanchon F. Finucane, MHS,[b] Millicent Higgins, MD,[c] and Joel C. Kleinman, PhD[b]

Bethesda and Hyattsville, Maryland

A national sample of 1944 white menopausal women ≥55 years old from the epidemiologic follow-up of participants in the National Health and Nutrition Examination Survey was reviewed to investigate the role of hormone therapy in altering the risk of death from cardiovascular disease. Women in the study were observed for up to 16 years after the baseline survey in 1971 to 1975. By 1987 631 women had died; 347 of these deaths were due to cardiovascular disease. History of diabetes (relative risk, 2.38; 95% confidence interval 1.73 to 3.26), previous myocardial infarction (relative risk, 2.12; 95% confidence interval 1.56 to 2.86), smoking (relative risk, 2.18; 95% confidence interval, 1.69 to 2.81), and elevated blood pressure (relative risk, 1.49; 95% confidence interval, 1.14 to 1.94) were strong predictors of cardiovascular disease–related death in this cohort. After adjusting for known cardiovascular disease risk factors (smoking, cholesterol, body mass index, blood pressure, previous myocardial infarction, history of diabetes, age) and education, the use of postmenopausal hormones was associated with a reduced risk of death from cardiovascular disease (relative risk, 0.66; 95% confidence interval, 0.48 to 0.90). The same protective effect provided by postmenopausal hormone therapy was seen in women who experienced natural menopause (relative risk, 0.69; 95% confidence interval, 0.45 to 1.06). (AM J OBSTET GYNECOL 1991;164:489-94.)

Key words: Postmenopausal, hormones, cardiovascular, mortality

Whether the administration of estrogen replacement therapy after menopause provides some degree of cardiovascular protection is currently a subject of debate. Cardiovascular disease is a leading cause of death among women. However, the age-specific death rates for cardiovascular disease among women are substantially less than those of men the same age. The fact that the disparity is less pronounced after age 60 has led some investigators to conclude that the protective effect is due to the production of endogenous estrogens and/or other functions of the ovaries. Furthermore, it has been hypothesized that the use of exogenous estrogen after menopause will sustain this advantage at the older ages. Several studies have shown that women who undergo estrogen replacement therapy enjoy a substantial advantage with regard to fewer cardiovascular events,[1-6] whereas other studies show little or no effect of estrogen replacement,[7, 8] and in some cases an increase in cardiovascular disease is demonstrated.[9, 10] The mortality studies[1-6] are based on a small number of deaths, primarily because of short follow-up periods.

A sufficiently large cohort with adequate follow-up is required to conclusively resolve the issue of the putative protective effect of hormone replacement therapy in menopausal women. The National Health and Nutrition Examination Survey (NHANES I) Epidemiologic Follow-up Study provides information on such a cohort. This cohort consists of a nationally representative sample of women from the United States, who, after >15 years of observation, have experienced the largest number of cardiovascular disease–related deaths reported to date in a prospective study. Furthermore, the study was conducted in an era in which the prescribing practice for estrogen replacement consisted almost exclusively of conjugated equine estrogen (Premarin).[11] This cohort is used to investigate the influence of postmenopausal hormone therapy on car-

From the Contraceptive Evaluation Branch, National Institute of Child Health and Human Development, Bethesda,[a] the Division of Analysis, National Center for Health Statistics, Hyattsville,[b] and the Epidemiology and Biometry Program, National Heart, Lung and Blood Institute, Bethesda.[c]
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Reprint requests: Pamela H. Wolf, DrPH, Office of Population, Agency for International Development, SA-18, Washington, DC 20523-1819.
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diovascular disease mortality and to study whether this effect varies with the type of menopause.

Material and methods

Study population. The NHANES I Epidemiologic Follow-up Study is a longitudinal study that is designed to investigate the relationships among clinical, nutritional, and behavioral factors measured at the NHANES I in 1971 to 1975 and to examine subsequent morbidity and mortality among study participants. The study attempted to trace and reinterview adult subjects (or their proxies), who ranged in age from 25 to 74 at the NHANES I (baseline). Hospital and nursing home records were collected to determine any overnight stays that had occurred since the baseline examination. Death certificates were obtained for the decedents. More detailed discussions of the design, operation, and content of both the NHANES I and the Epidemiologic Follow-up Study are described elsewhere.[12]

The first wave of the follow-up, which was conducted from 1982 to 1984, resulted in information on nearly 93% of the follow-up group. Additional waves were conducted in 1986 (for those at least 55 years old at baseline) and again in 1987. The analysis reported here is restricted to white female subjects in the NHANES I Epidemiologic Follow-up Study who were at least 55 years old and menopausal at the time of the baseline survey. More than 96% of these eligible women were successfully traced at some time during the follow-up. Only women who had complete information on the cardiovascular risk factors under study, including the use of postmenopausal hormones, were considered in this analysis.

Risk factors. For the purpose of this study, menopause is defined as the complete cessation of menstruation as reported at baseline; timing of menopause is based on baseline information concerning whether menses ceased before age 45 (early menopause). Type of menopause was categorized as natural or surgical, on the basis of information obtained in the inverview. Cardiovascular risk profiles at baseline were defined according to the following criteria. Smoking status at baseline was determined either directly from the baseline questionnaire or from the lifetime smoking history taken at the first follow-up interview, or it was imputed in the case of missing information. Women were categorized as smokers if they reported at baseline that they were currently smoking. High cholesterol was defined according to the 1984 National Institutes of Health Consensus Development Conference (>260 mg/dl).[13] Body mass index in metric units is computed as weight [kilograms]/(height[meters])^2 and is categorized into quartiles on the basis of all women who were menopausal at baseline. High blood pressure is defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg.[14] Prevalence of diabetes at baseline and history of myocardial infarction were based on self-reported data. Women were categorized as either ever-users or nonusers of postmenopausal hormones, on the basis of questions asked in the 1982 to 1984 questionnaire. Since this information was obtained by self-report, it is subject to some recall bias. In addition, a proxy respondent provided the information on hormone use for subjects that were deceased or incapacitated at the time of the 1982 to 1984 interview. The complexities associated with use of proxy information are addressed below.

Cardiovascular disease mortality. Vital status was ascertained in the 1982 to 1984, 1986, and 1987 waves of the follow-up. Mortality information was obtained from either a death certificate and/or a proxy respondent. The underlying cause of death was coded by rules of the *International Classification of Diseases*, 9th revision (ICD-9), for the 96.6% of the decedents in this menopausal cohort with an available death certificate. In this study mortality from cardiovascular disease is defined as underlying cause of death coded to ICD-9 390-459. Decedents with no death certificate (3.4%) were omitted from the analysis.

Statistical analysis. Relative risks were estimated with the Cox proportional hazards model and the PHGLM procedure in the SAS software system.[15] This procedure takes into account the variable length of follow-up, which is computed from the time of the baseline survey to the last known date alive and which ranged from 11.4 to 16.3 years among the survivors (average, 14.1 years) and 2 months to 16.3 years among the decedents (average, 8.6 years). First, we computed the estimated relative risks for known cardiovascular risk factors (smoking, elevated cholesterol, high body mass index, elevated blood pressure, history of diabetes, previous myocardial infarction, age at examination), age at menopause, and use of hormones for treatment of menopause. Since estrogen depletion is more abrupt and since the use of hormones is more common after surgical menopause, it was of interest to specifically evaluate the role of hormone replacement after natural menopause, when estrogen replacement is optional and is often determined by the level of discomfort of the patient. Because hormone use may be related to health care access and utilization, which are greater among women of higher socioeconomic status, all models included education as a measure of socioeconomic status.

Two potential sources of bias that might affect these analyses were investigated. Bias might have been introduced by elimination of subjects with missing or unknown hormone use data (12%). To examine this, we reanalyzed the data by imputing hormone use for missing values. Three imputed values were created accord-

ing to the following criteria: (1) women with unknown estrogen status were as likely to use estrogen as were women with know estrogen status; (2) estrogen use was twice as likely to occur among the unknown as the known; (3) estrogen use was one half as likely to occur among the unknown as the known. All three imputations were applied within age-at-examination strata. Bias also might have been introduced by allowing proxy responses to the hormone question. To evaluate this particular bias, relative risk estimates were recomputed for subjects who survived to the 1982 to 1984 interview and who responded to the interview themselves. This precaution eliminated all proxy respondents from the study.

Results

A total of 2461 white female subjects from the NHANES I Epidemiologic Follow-up Study were identified as postmenopausal and at least 55 years old at baseline. Subjects were eliminated from final analysis because of unknown age at menopause ($n = 62$), inability to determine cardiovascular risk status at baseline ($n = 90$), unknown level of education ($n = 10$), loss to follow-up ($n = 71$), and missing information on hormone use at the 1982 to 1984 follow-up ($n = 284$). Table I shows the characteristics of the remaining 1944 women. The mean age of the cohort at baseline (1971 to 1975) was 65.7 years. Nearly 19% of the women were smokers, almost 40% had elevated cholesterol levels, and 67.7% had elevated blood presure. Overall, 62.4% began follow-up with two or more known cardiovascular disease risk factors. By the initial recontact from 1982 to 1984, 367 deaths (18.9%) had occurred. An additional 264 deaths occurred between 1982 to 1984 and 1987. Overall, 347 of the 631 deaths resulted from cardiovascular disease.

The characteristics of the cohort by hormone use and type of menopause are given in Table II. There were 1377 women (70.8%) who experienced natural menopause, 430 women (22.1%) who reported a surgically induced menopause, and 137 women (7.1%) for whom the type of menopause could not be determined. The table is based on the 1807 women for whom information on the type of menopause was available. Hormone use was nearly three times higher in the surgical menopause group, as compared with use in women undergoing natural menopause (38.4% vs 15.5%, respectively). Hormone users in general had lower blood pressure, lower prevalence of diabetes, and lower body mass index at baseline. On the other hand, when compared with nonusers, women using postmenopausal hormones had a higher prevalence of smoking. Cholesterol levels and history of myocardial infarction were the same for both hormone users and nonusers. The risk factor profile of women undergoing natural menopause was virtually the same as that of the surgical menopause group, although the surgical group had a slightly higher percentage of women with a history of previous myocardial infarction (7.9% vs 6.8%). Over half of the women in the sample had two or more cardiovascular risk factors present at baseline, regardless of type of menopause and hormone use history; only 8% were free of the risk factors under study.

Table I. Baseline cardiovascular disease risk factor profiles for white menopausal women, aged ≥55 (NHANES I Epidemiologic Follow-up Study, $N = 1944$)

Smoking (%)	18.6
Elevated cholesterol*	38.7
High body mass index† (%)	25.4
Elevated blood pressure‡ (%)	67.7
Diabetes (%)	7.6
History of myocardial infarction (%)	7.4
≥2 Risk factors (%)	62.4
Deaths (No.)	
Total	631
Cardiovascular disease–related	347
Age at examination (yr, mean)	65.7

*As defined by 1984 National Institutes of Health Consensus Development Conference (>260 mg/dl).

†>29.5.

‡Systolic blood pressure >140 mm Hg or diastolic pressure >90 mm Hg.

When multivariate risk estimates for cardiovascular disease mortality were generated for the cohort (Table III), age, history of diabetes, smoking, and elevated blood pressure were strong predictors. Individuals in the third quartile of body mass index (25.5 to 29.4) experienced a reduced risk of cardiovascular disease–related death, as compared with the risk in individuals in the lowest quartile. Elevated cholesterol, early menopause (age <45), and education did not alter cardiovascular disease–related mortality risk for women in this cohort.

Hormone use was associated with a 34% reduction in cardiovascular mortality risk (relative risk, 0.66; 95% confidence interval, 0.48 to 0.90) after adjusting for known cardiovascular risk factors and education. A separate model for natural menopause was generated to investigate whether elective use of hormones provides the same level of protection. The adjusted relative risk for hormone use among women experiencing natural menopause was 0.69 (95% confidence interval, 0.45 to 1.06) (Table IV). This effect was smaller in the surgical menopause group (not shown) for whom the estimate of cardiovascular disease–mortality risk was 0.80 (95% confidence interval, 0.39 to 1.67). The confidence intervals surrounding this estimate are wider, because of

Table II. Baseline cardiovascular disease risk factor profiles for white menopausal women, aged ≥55, by type of menopause and hormone use (NHANES I Epidemiologic Follow-up Study, $N = 1807$)

	Natural			*Surgical*		
	Users ($n = 214$)	*Nonusers ($n = 1163$)*	*All natural ($n = 1377$)*	*Users ($n = 165$)*	*Nonusers ($n = 265$)*	*All surgical ($n = 430$)*
No. of deaths						
Total	41	414	455	23	40	63
Cardiovascular disease–related	24	235	259	12	25	37
Age (yr, mean)	63.9	66.3	66.0	63.2	65.0	64.3
Risk factor						
Smoking (%)	22.0	18.2	18.8	27.3	14.7	19.5
Elevated cholesterol* (%)	38.8	38.7	38.7	41.8	42.3	42.1
High body mass index† (%)	15.4	28.0	26.0	16.9	27.9	23.7
Elevated blood pressure‡ (%)	54.7	70.3	67.9	64.2	71.7	68.8
Diabetes (%)	4.2	7.3	6.8	6.1	9.0	7.9
History of myocardial infarction (%)	6.5	6.9	6.8	8.5	7.5	7.9
≥2 Risk factors (%)	54.7	64.7	63.2	59.4	64.9	62.8

*As defined by 1984 National Institutes of Health Consensus Development Conference (>260 mg/dl).
†>29.5.
‡Systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg.

both the small number of women undergoing surgical menopause ($n = 430$) and the few cardiovascular disease deaths ($n = 37$) in this group.

To investigate the effect of proxy responses, the estimates were recomputed for the subgroup of women who survived until the 1982 to 1984 interview. This analysis is based on the 135 cardiovascular disease deaths that occurred between 1982 and 1987. This eliminates proxy responses to the hormone questions. The overall relative risk for cardiovascular disease mortality among hormone users in this group of menopausal women was consistent with a protective effect although to a lesser degree (relative risk 0.80; 95% confidence interval, 0.50 to 1.26), as was the estimate for women experiencing natural menopause (relative risk 0.80; 95% confidence interval, 0.44 to 1.52). The confidence intervals for both estimates are much wider. The relative risk estimates that were generated with the random assignment of the estrogen variable for missing data (not shown) did not differ substantially from those given in Tables III and IV. These results indicate that two potential sources of bias (missing data and proxy responses) do not appear to have affected the findings presented in this investigation.

Comment

The current study demonstrates a reduction in cardiovascular disease–related mortality among women who use postmenopausal hormones. Relative to women who had never taken hormones, the reduction in risk is 34%. This protective effect is not explained by known or suspected cardiovascular risk factors or by the timing of the menopause, and the effect persists despite the prevalence of two or more cardiovascular disease risk factors at baseline in half the hormone users. We have attempted to examine two potential sources of bias (missing hormone use information and the use of proxy responses) by performing additional analyses in which we imputed the hormone use data on the basis of three different assumptions and restricted the analysis to subject reponses only. Although the resultant analyses yielded wider confidence intervals, they also indicated a protective effect.

It is important to recognize that in many observational studies users and nonusers of estrogen differ at baseline with respect to important cardiovascular risk factors such as smoking, body mass index, and blood pressure. We have attempted to adjust for differences in cardiovascular disease risk factors by including them in the statistical model.

It should be noted that the scope of cardiovascular disease mortality in this study is much broader than that in earlier mortality studies and that this study includes death from cerebrovascular disease. Nevertheless, the results are similar in the magnitude of the protective effect of postmenopausal estrogens found in

Table III. Multivariate risk estimates for cardiovascular disease–related mortality among 1944 white menopausal women, age ≥55 at baseline, NHANES I Epidemiologic Follow-up Study

Risk factor	*Relative risk*	*95% Confidence interval*
Smoking	2.18	1.69-2.81
Elevated cholesterol (≥260 mg/dl)	0.95	0.76-1.18
Body mass index* (weight/height^2)		
2nd quartile (22.5-25.4)	0.77	0.56-1.06
3rd quartile (25.5-29.4)	0.71	0.52-0.96
4th quartile (>29.5)	0.92	0.68-1.25
Elevated blood pressure (systolic >140 and diastolic >90 mm Hg)	1.49	1.14-1.94
Diabetes	2.38	1.73-3.26
Previous myocardial infarction	2.12	1.56-2.86
Age†	1.87	1.82-1.91
Hormone use	0.66	0.48-0.90
Early menopause (<45 yr)	0.99	0.78-1.27
Education‡	0.83	0.66-1.05

*Comparison with first quartile (<22.5).

†Five-year increments in age.

‡Comparison with <12 years completed.

studies limited to ischemic heart disease deaths. Thus our findings suggest that the protective effect of postmenopausal hormones extends beyond that provided for ischemic heart disease.

Of the cohort studies to date that have examined the relationship between postmenopausal hormone use and cardiovascular disease,[3-6, 8, 9] most have found estrogen to be protective. However, two of the larger prospective studies report contradictory findings. The Nurses Cohort Study[3] showed that estrogen replacement therapy reduced the risk of severe coronary heart disease and that the increase in cardiovascular disease associated with surgical menopause could be prevented by estrogen replacement therapy. However, the Framingham study[9] suggested that women who used postmenopausal estrogen were subject to an increase in the development of cardiovascular disease and indicated that rates of myocardial infarction were increased among estrogen users who smoked, whereas cerebrovascular disease rates were increased among nonsmokers. In subsequent analyses[16] of the Framingham cohort postmenopausal estrogen use was not related to the incidence of serious coronary disease among women aged 50 to 59, but an association among women aged 60 to 69 was noted. Despite the large cohort sizes and ample follow-up periods, both these studies generated few cases of cardiovascular disease, in part because of the age of the women at entry into the study. In contrast, this investigation is a large, heterogeneous, national sample followed up for a maximum of 16 years, which has yielded the largest number of cardiovascular disease–related deaths reported in a prospective study to date.

Although the use of postmenopausal estrogens declined to some degree in the mid-1970s, when it was associated with an increase in endometrial cancer,[17] the use of estrogens has increased in recent years, although they are administered at lower prescribed doses and are often administered with a progestational agent. A recent survey of gynecologists[11] showed that 94% routinely administer estrogens for postmenopausal therapy, which shows an increase from 79% 10 years ago.

Table IV. Relative risk of cardiovascular disease–related mortality for white women using postmenopausal hormones, compared with that for nonusers, for total cohort and for women experiencing natural menopause, by type of response from women aged >55 and menopausal at baseline (NHANES I Epidemiologic Follow-up Study)

	Relative risk, †*	*95% Confidence interval*
Total cohort		
Subject and proxy responses (n = 1944)	0.66	0.48-0.90
Subject responses only (n = 1503)	0.80	0.50-1.26
Natural menopause		
Subject and proxy responses (n = 1377)	0.69	0.45-1.06
Subject responses only (n = 1059)	0.82	0.44-1.52

*Hormone users as compared with nonusers.

†Adjusted for age, smoking, blood pressure, cholesterol, body mass index, diabetes, previous myocardial infarction, and education, with use of the Cox proportional hazards model.

More than 2 million women are currently using estrogen for noncontraceptive reasons each year.[18]

Hormone therapy is one of the possible interventions that can improve a woman's cardiovascular status. The findings presented here indicate that prevention of 20% to 35% of the cardiovascular deaths experienced by women who do not use estrogen is possible. This benefit must be weighed against the potential risks, such as gallbladder disease, high blood pressure, thromboembolic disease, impaired glucose tolerance, and endometrial cancer, which are associated with estrogen therapy.[19] However, the combined incidence rates of these adverse effects are exceeded by that of heart disease.

Although the baseline and 1982 to 1984 questionnaires do not explicitly acquire information about specific estrogen preparation used, Premarin was used in 95% of the estrogen therapies; that use has remained constant during the last decade. Current prescribing practices indicate a trend toward combined therapy. Whether the addition of a progestational agent will diminish the beneficial effects of estrogen is unknown. New questions added to the most recent interview of this cohort in 1987 addressed issues of dose and the addition of a progestational agent. This analysis used a limited definition of hormone use (ever-use vs never-use). Future analyses will incorporate additional aspects of patterns of use, such as timing and duration, to further specify the nature of the relationship between hormone use and cardiovascular disease mortality. Clinical trials are also needed to investigate new preparations of estrogen and progestational agents for hormone replacement, as well as to determine alternative routes of administration such as patches and implants.

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Endosalpingosis as a cause of chronic pelvic pain

Susan A. Davies, MD, and Victoria M. Maclin, MD
Chicago, Illinois

Endosalpingosis, a condition characterized by ectopic oviduct epithelium, is diagnosed histologically by the appearance of benign ciliated and nonciliated columnar cells in an abnormal location. Endosalpingosis is typically without symptoms. Our unusual case report shows symptomatic endosalpingosis first seen with chronic pelvic pain. Various aspects of this disease will also be discussed. (Am J Obstet Gynecol 1991;164:495-6.)

Key words: Endosalpingosis, pelvic pain, ectopic tubal epithelium

Endosalpingosis is a condition characterized by ectopic oviduct epithelium.[1] Typically endosalpingosis is diagnosed histologically by the appearance of benign ciliated and nonciliated columnar cells, along with peg cells, in an abnormal location.[1] Macroscopically this disease is usually not detectable. When visually obvious, endosalpingosis can be seen as multiple, punctate (1 to 2 mm), white to yellow, opaque or translucent, fluid-filled, cystic lesions that grossly impart a granular appearance to the involved peritoneal area as exemplified by the following case report.[2] Rarely, larger cysts may be present.

Case report

A 19-year-old white gravida 0 female was first seen with pelvic pain. Her pain had progressed since menarche from dysmenorrhea to occasional intermenstrual pain to virtually constant pain. She began menarche at age 14 years. Severe menorrhagia and dysmenorrhea resulted in her being placed on oral contraceptives soon after menarche; however, oral contraceptives were discontinued by age 15 years because of depression. Cessation of oral contraceptives resulted in resumption of progressive dysmenorrhea.

Multiple medical opinions were sought for pain relief. In November 1988 she underwent diagnostic laparoscopy. Biopsy specimens of the pelvic peritoneum revealed "a few ciliated polygonal-epithelial lined cysts with a focal underlying endometrial-type stroma consistent with endometriosis foci." Subsequently, she underwent 6 months of Danocrine therapy for suppression of endometriosis. Dosage during the first 5 months was 800 mg per day. However, during the sixth month, because of persistent vaginal bleeding and despite having achieved pain relief, her dose was increased to 1000 mg per day. Discontinuation of therapy was followed by rapid resumption of pelvic pain, leading to a second laparoscopy at another institution in May 1989. Pelvic inspection at that time revealed "miliary dissemination of minute particles and nodules between 0.5 mm and 2 mm in diameter." These biopsy specimens were histologically described as "atypical cysts, rule out ruptured serous cystadenoma of ovary," despite normal appearance of the ovaries at the time of laparoscopy.

The patient was referred to Rush–Presbyterian–St. Luke's Medical Center, Section of Gynecologic Oncology, where slides from the previous biopsy specimens were reviewed and a diagnosis of endosalpingosis was made. She was referred in August 1989 to the Section of Reproductive Endocrinology for hormonal suppression with a gonadotropin-releasing hormone agonist. She reported a progressive decrease in pain during the previous month. A urinary β-human chorionic gonadotropin test was performed (because of persistent amenorrhea during the 4 months since discontinuation of Danocrine therapy) and was positive. Pelvic examination revealed a 7-week-size uterus and vaginal ultrasonography indicated a 7½–week intrauterine pregnancy. In view of her pregnancy and decreasing pelvic pain, no medication was prescribed.

Comment

Endosalpingosis was first described in 1930 by Sampson.[3] His study of 100 cases of tubal stumps after tubal ligations led to the observation that sprouts of fallopian tube epithelium often invade the wall of the stump and may even extend beyond it after healing is complete.[3] Endosalpingosis is typically located in the visceral pelvic peritoneum covering the uterus, fallopian tubes, ovaries, and cul-de-sac. Less frequent locations involve the pelvic parietal peritoneum, omentum, bladder and bowel serosa, periaortic area, and within the skin.[2] Endosalpingosis is commonly associated with previous tubal surgery such as tubal sterilizations and salpingectomies and is also associated with chronic salpingitis.[1] Furthermore, it is usually a process without symp-

From the Department of Obstetrics and Gynecology, Rush–Presbyterian–St. Luke's Medical Center.
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Reprint requests: Victoria M. Maclin, MD, Section of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Rush–Presbyterian–St. Luke's Medical Center, 1653 W. Congress Parkway, Chicago, IL 60611.
6/1/25746

toms, noted incidentally by a pathologist on a histologic specimen. It is of interest that symptomatic endosalpingosis (unlike the condition without symptoms) is associated with psammoma body formation.[2]

The various hypotheses as to the cause of endosalpingosis are: (1) transplantation, tubal mucosa is transplanted to peritoneal surfaces, usually during surgery; (2) direct extension, tubal epithelium extends to the peritoneum as exhibited by adhesion formation; (3) reactive, salpingitis causes tubal epithelium to proliferate with repair being the goal and retrograde spill results; (4) coelomic metaplasia, coelomic peritoneum cells are multipotential and can differentiate into oviduct epithelium; and (5) lymphatic-vascular spread.[2]

It is important to rule out endosalpingosis when endometriosis is being considered because both have been reported in the same person. Endometriosis is histologically characterized by the presence of endometrial glands and stroma. Furthermore, endometriosis often has a hemorrhagic appearance as a result of this tissue's response to hormonal stimuli. Endosalpingosis, as stated previously, is histologically characterized by the appearance of benign ciliated and nonciliated columnar cells in an abnormal location. Endosalpingosis does not exhibit a hemorrhagic response to hormonal stimuli, which further helps in the differentiation of this condition from endometriosis. Also metastasis from ovarian papillary serous tumors must be differentiated from endosalpingosis inasmuch as both may have psammoma bodies and endosalpingosis may form a papillary configuration as a result of irritation, trauma, or manipulation. Histologic features that support the diagnosis of metastasis from an ovarian papillary serous tumor include the presence of an infiltrative pattern, a desmoplastic stromal response, the presence of only one cell type, and the absence of a basement membrane surrounding the glands.[2] The latter morphologic features do not characterize benign endosalpingosis.

In summary, endosalpingosis is a benign disease that is diagnosed histologically by the presence of ectopic oviduct epithelium. Our patient exhibits several unusual aspects of this process. First, the extent of the disease is evident macroscopically with the apparent miliary dissemination. Also, our patient is symptomatic, contrary to the usual presentation without symptoms. Although the cause of this disease remains unknown, it is likely that several of the proposed hypotheses may actually play a role. To date there is no known treatment protocol for endosalpingosis, but hormonal suppression regimens commonly used for endometriosis may be of benefit in this special group of patients as exemplified by our case report.

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Transvaginal ultrasonographic definition of the central nervous system in the first and early second trimesters

Ilan E. Timor-Tritsch, MD, Ana Monteagudo, MD, and Wendy B. Warren, MD
New York, New York

A 6.5 MHz transvaginal sector scanner was used to describe the fetal central nervous system anatomy from 6 to 14 weeks. One hundred thirty-three pregnant patients were scanned after the crown-rump measurements were found to be within a range of ≤4 days from menstrual age. The number of possible ultrasonographic sections ("slices"), as well as the number of successful views in the axial, coronal, and sagittal sections, was tabulated. A detailed list of central nervous system structures that are seen at each gestational age is presented. The results showed that a significant number of brain structures could be imaged. The number and the clarity of the structures and the number of successful views in the three scanning planes increased with gestational age. We concluded that transvaginal scanning for central nervous system maldevelopment can be started in the late first and early second trimesters. (Am J Obstet Gynecol 1991;164:497-503.)

Key words: Transvaginal ultrasonography, central nervous system, fetus, first trimester, second trimester

High-frequency transvaginal ultrasonography has been shown to be useful in imaging early pregnancy.[1-4] Previous work has been published which stresses its potential use in the malformation workup of the first and early second trimesters.[5, 6] To facilitate the proper clinical application of transvaginal ultrasonography from 6 to 14 weeks, a study that describes the chronologic appearance of normal central nervous system structures was undertaken.

Patients and methods

Patients. Transvaginal ultrasonography was performed on 133 women with low-risk pregnancies and singleton fetuses. Only one scan for each fetus was used for the purposes of this study.

Dating of the gestation. The gestational age by menstrual history was compared with the gestational age by the crown-rump length.[7] Only those cases with a discrepancy that was ±5 days were included in the study. In 98 of the 133 cases this discrepancy was <3 days. The distribution of the patients by gestation is shown in Table I.

Equipment. A single-element vaginal sector scanner probe, which operated at 6.5 MHz, was used (Elscint, Inc., Haifa, Israel).

Scanning planes attempted. Sagittal, axial, and coronal planes of the fetus were sought. A note was made

Table I. Central nervous system study: Distribution by age

Weeks	*No.*
6	9
7	12
8	15
9	17
10	16
11	17
12	15
13	14
14	18
TOTAL	133

Table II. Number of sections obtained in each plane

Weeks	*No.*	*Axial*	*Coronal*	*Sagittal*
6	9	0	1	1
7	12	1	1	1
8	15	2	2	1-2
9	17	2-3	2-3	3
10	16	3	3-4	3
11	17	4	4	3
12	15	4-5	4-5	3
13	14	5+	5+	4-5
14	18	5+	5+	5+

From the Ultrasound Division, Department of Obstetrics and Gynecology, Sloane Hospital for Women, Columbia Presbyterian Medical Center.
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Reprint requests: Ilan E. Timor-Tritsch, MD, Department of Obstetrics and Gynecology, Columbia Presbyterian Medical Center, 622 West 168th St., New York, NY 10032.
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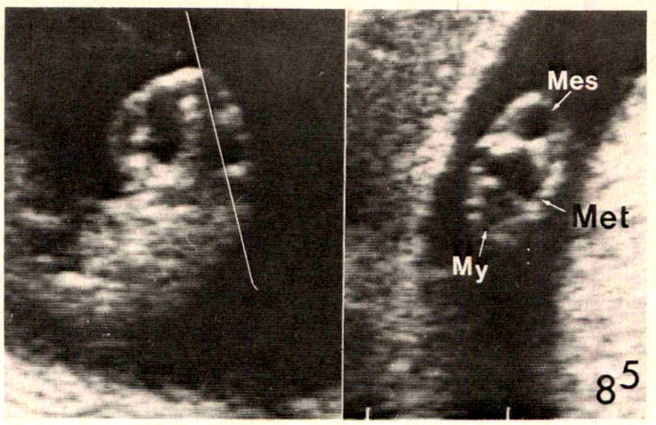


Fig. 1A. Midsagittal section at 8 weeks 5 days. The sagittal arrangement of the brain cavities is represented by the sonolucent structures. On the *right*, a coronal section is shown. This was obtained by scanning along the section line that is shown on the sagittal view. Structures seen were: the mesencephalon *(Mes)*, metencephalon *(Met)*, and myelencephalon (My).

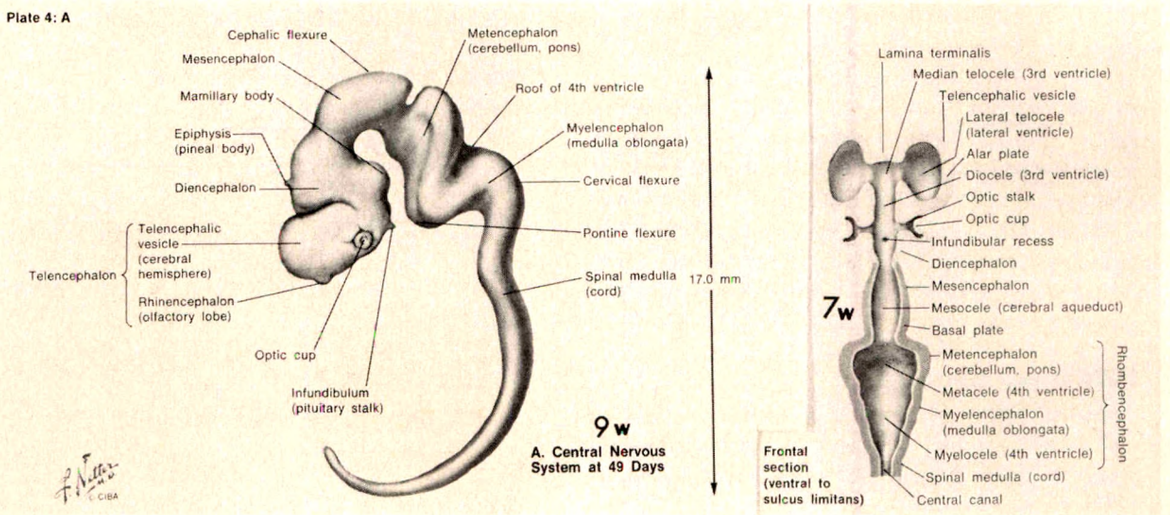


Fig. 1B. For comparison, drawings of the central nervous system of the fetus at 9 and at 7 menstrual weeks are presented. (From Crelin ES. Development of the nervous system. Clin Symp 1974:26: 1-32. Copyright 1974 Ciba-Geigy Corporation. Reproduced with permission from the *Clinical Symposia*, Frank H. Netter, MD, and E. S. Crelin, MD. All rights reserved.)

Table III. Number of successful views in various scan planes as a function of gestational age

Weeks	*No.*	*Axial*	*Sagittal*	*Coronal*	*Spine*
6	9	?	8	6	4
7	12	2	11	11	9
8	15	5	13	13	10
9	17	14	15	13	8
10	16	13	11	15	13
11	17	14	10	15	11
12	15	14	15	14	14
13	14	14	14	12	12
14+	18	17	18	18	18

of the number of subsequent sections that could possibly be studied for each fetus.

Successful views across each plane. For each gestational age group, we recorded the number of cases in which a structure was adequately evaluated.

Correlation. Correlation of the detected structures with the appropriate structure in embryology textbooks was also attempted for each gestational age.[8-10]

It is beyond the limits of this article to present images of all of the detected structures throughout each week of gestation. Only a small number of selected images are included in this section.

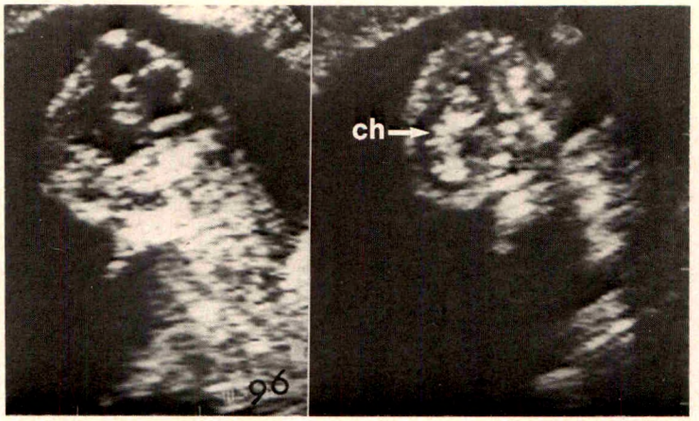


Fig. 2. At 9 weeks 6 days a midsagittal section reveals more detail of the convoluted cavities of the brain *(left side)*. The parasagittal section shows the lateral ventricle hosting the hyperechoic choroid plexus *(ch) (right side)*.

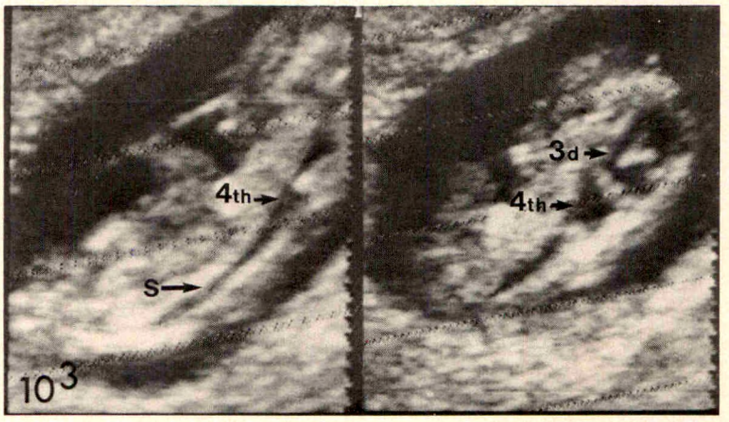


Fig. 3. Two subsequent coronal sections of the fetus at 10 weeks 4 days, which demonstrate the spinal canal *(S)*, fourth ventricle *(4th)*, and third ventricle *(3d)*.

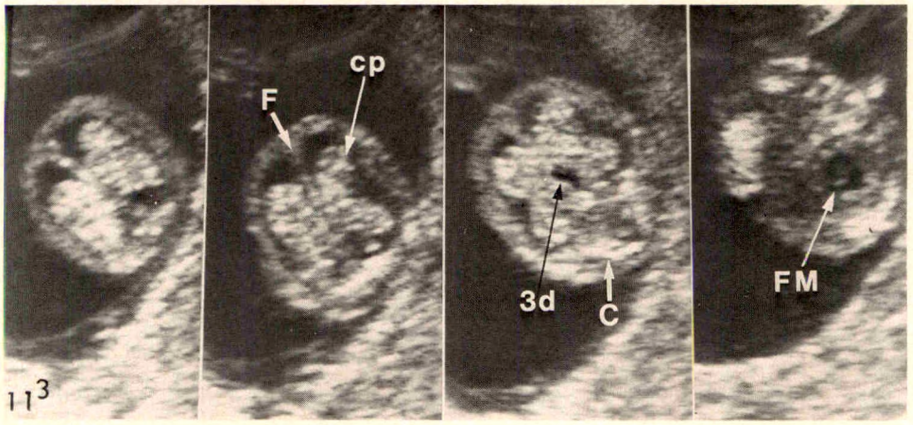


Fig. 4. Four serial axial sections at 11 weeks 3 days, starting at the top *(left)* and ending with the one taken at the base of the skull. Structures seen are the falx *(F)*, choroid plexus *(cp)*, third ventricle *(3d)*, cerebellum *(C)*, and foramen magnum *(FM)*.

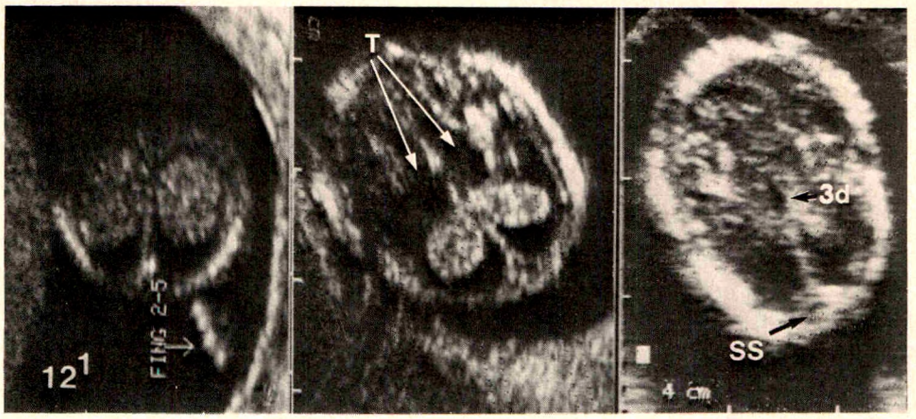


Fig. 5. At 12 weeks 1 day the coronal section (left) shows the falx and choroid plexus. The axial sections (*middle* and *right*) reveal the hyperechoic choroid plexus on top of the thalami *(T)* in the lateral ventricles. Only a thin cortical mantle is still seen. The third ventricle *(3d)* and the frontal part of the sagittal sinus *(SS)* are also seen.

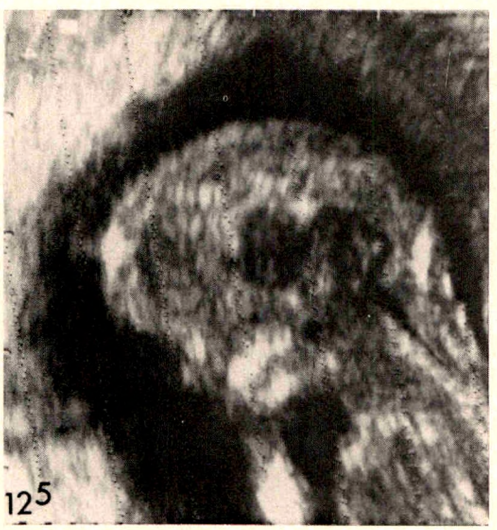


Fig. 6. A midline sagittal section at 12 weeks 5 days shows the sonolucent convolutions of the ventricular system leading into the spinal canal.

Results

The number of sections that were obtained in each plane from 6 to 14 weeks is shown in Table II. During the time period of 6 weeks 0 days to 7 weeks 6 days, only one significant plane could be obtained. At 8 to 10 weeks two to three sections were obtained, and after 11 weeks four, five, or even more sections could be produced through each scanning plane.

In the sagittal plane we obtained a lower number of scanning sections than in the coronal or axial planes because of the smaller transverse dimensions of the embryo-fetus.

The number of successful views in the three scanning planes (axial, coronal, sagittal) increased with gestational age. After the tenth week, almost all three of the scanning planes were obtained (Table III). The structures that were detected throughout each week of gestation from 6 to 14 weeks are detailed here.

6 weeks 0 to 6 days. Only one section through each plane was obtained. No brain structures were imaged. However, sections of the neural tube were seen on the coronal plane.

7 weeks 0 to 6 days. The telencephalic vesicle and mesencephalic vesicles were seen. On the axial and coronal planes, the myelocele was seen close to the end of this week.

8 weeks 0 to 6 days. In addition to the previous structures, the typical convoluted arrangement of the fetal brain in the sagittal plane becomes evident (Fig. 1A). The forebrain vesicles (telencephalic, diencephalic, and mesencephalic) were imaged. In addition to these, on the coronal section, the cerebral aqueduct, the metacoele (future fourth ventricle), and the myelocele (lower funneling of the fourth ventricle) were evident. On an axial section, the roof and cavity of the future rhombencephalon could be viewed. The posterior contours of the body were discernible. Fig. 1B enables comparison of the above-mentioned structures to drawings that depict the brain structures in question.

9 weeks 0 to 6 days. The outline of the ventricular system along the sagittal midline became clear (Fig. 2). The lateral telocoele (lateral ventricles) became distinct for the first time along with the sonographic markers of this age, the echogenic choroid plexi. In a few cases the thalami were seen. At this gestational age, multiple sections could be obtained.

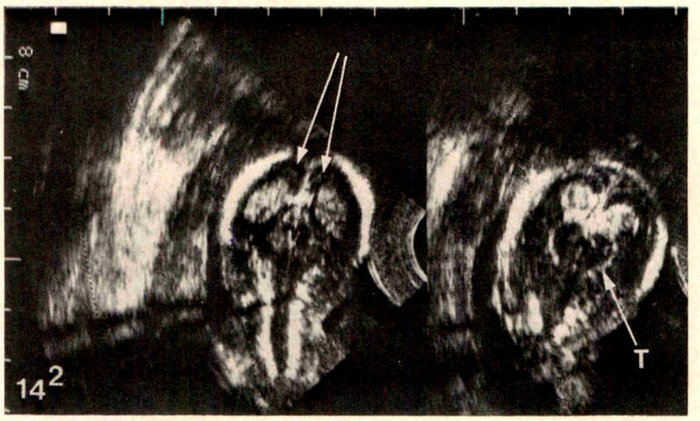


Fig. 7. Two successive coronal sections at 14 weeks 2 days. The left one is a posterior section that shows the choroid plexus filling the occipital horn of the lateral ventricles. A cortical mantle of 2 to 3 mm is now seen (marked by two *long arrows*). The section on the right shows the thalami *(T)*. The hyperechoic choroid plexi are on top of the thalami.

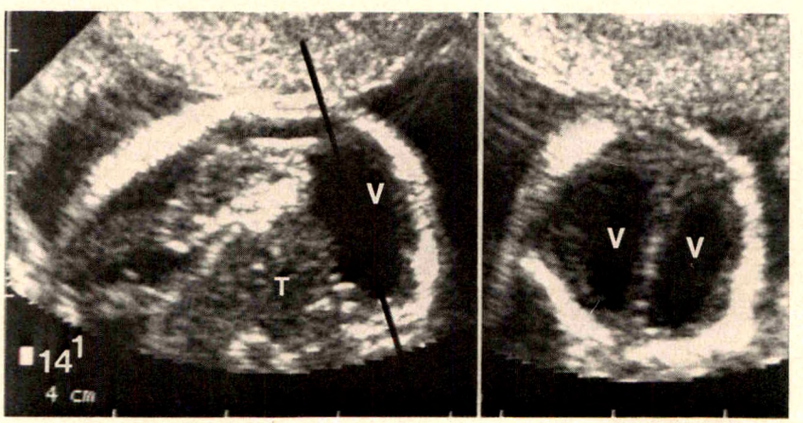


Fig. 8. A parasagittal section *(left)* through the brain at 14 weeks 1 day clearly shows the hyperechoic choroid plexus on top of the thalamus *(T)* which leaves a sonolucent frontal horn of the lateral ventricle *(V)* with ample "free space." The coronal section across the *black line* shows the typical frontal horns *(V)*.

10 and 11 weeks. The third and fourth ventricles could clearly be seen on the coronal section (Fig. 3). Clear choroid plexi were imaged on top of the thalami. Lateral ventricles with the choroid plexi and the cerebellum were seen on a small number of axial sections; close to the base of the skull the foramen magnum was consistently seen (Fig. 4).

12 weeks 0 to 6 days. The cerebellum, thalami, and eyes were better defined than previously. The third ventricle and choroid plexi were clearly defined (Fig. 5). The midline sagittal section showed for the last time the convoluted ventricular system (Fig. 6). The spinal contours were crisply outlined.

13 weeks 0 to 6 days. Only a few new structures were seen; however, the ones that were seen previously were increasingly better defined. The two layers of the falx cerebelli and falx cerebri could be seen. The larger sagittal and coronal venous drainage systems were seen. The choroid plexus "retracted" from the frontal horn and was shown to be predominantly located in the occipital horn (its final normal position). The cortical mantle and white matter are thick enough to be imaged the first time.

14 weeks 0 to 6 days. The structures that were seen previously were all imaged; however, their detection was easier. Since at this gestational age the size of the

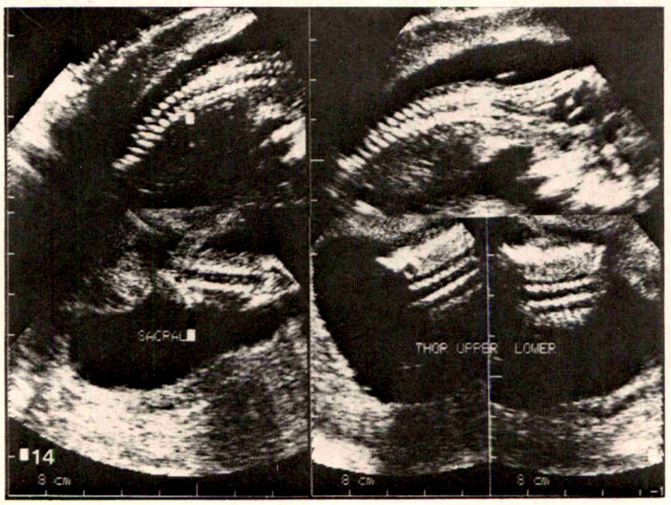


Fig. 9. Imaging of the spine in the sagittal plane and in several coronal sections is shown at 14 weeks.

fetus approaches the imaging limits of the vaginal probe, one had to apply a gentle fundal pressure to move the area of interest into the focal range of the probe. Spontaneous change of the fetal position was also helpful. The coronal sections were usually clear (Fig. 7). The frontal horns of the lateral ventricles displayed prominent sonolucent spaces, which were "free" of the choroid plexi (Fig. 8).

Comment

The number of practitioners who use transvaginal ultrasonography is constantly on the rise. If the crispness of the images that are created during the first and the early second trimesters is considered, it seems natural that this easily mastered scanning method will find its way into most obstetricians' offices. Whether in private offices or at tertiary centers, increasingly more ultrasonographers and physicians will be confronted by images similar to the ones presented in this study. The mean of the various sonolucencies and structures of the central nervous system has to be clear to everyone who is engaged in imaging of the fetus by transvaginal ultrasonography.

It is imperative that the normal ultrasonographically imaged central nervous system structure be well defined if clinicians are to use transvaginal ultrasonography for the workup of a 10-week-old to 14-week-old fetus for anomalies. The study that is presented here may form the partial basis for other such studies.

Several basic phenomena were revealed by this study. First, and probably the most imporant, was that an increasing number of central nervous system structures could be imaged with increasing gestational age. The second phenomenon was that these structures correspond perfectly to the already well-documented embryonic-fetal central nervous system structures that are depicted in available textbooks. Any person who engages in the ultrasonographic imaging of the embryonic-fetal central nervous system should scrutinize these atlases and textbooks to avoid misdiagnoses.

The third phenomenon was the ability to obtain an increasing number of sections ("slices") through any of the three classic scanning planes, as well as the fact that the number of possible sections increased with the gestational age.

We believe that, on the basis of this study, there is a possibility to detect maldevelopment of the central nervous system with reasonable accuracy in the late first and early second trimesters.

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Endodermal sinus tumor of the ovary during pregnancy: A case report

A.G.J. van der Zee, MD,[a] H.W.A. de Bruijn, PhD,[a] J. Bouma, MD,[a] J.G. Aalders, MD,[a] J.W. Oosterhuis, MD,[c] and E.G.E. de Vries, MD[b]

Groningen, The Netherlands

Serum α-fetoprotein screening led to the detection of an endodermal sinus tumor of the ovary in a 24-year-old female in week 17 of pregnancy. After surgery, chemotherapy was postponed. In week 28 levels of serum α-fetoprotein increased, but delivery was delayed until 33 weeks' gestation. After delivery, the patient received four chemotherapy courses (cisplatin, etoposide, and bleomycin). Mother (24 months after last chemotherapy) and child are doing well. (Am J Obstet Gynecol 1991;164:504-6.)

Key words: Endodermal sinus tumor, pregnancy

Endodermal sinus tumor of the ovary is a rare, malignant germ cell tumor that primarily affects children and young women. The prognosis for these patients has improved since platinum-containing chemotherapy was added to surgery.

The association of endodermal sinus tumor and pregnancy is even more extraordinary. Knowledge of chemotherapy during pregnancy is growing, but limited data are available to determine the long-term risks for the in utero–exposed children. In this report we present a patient with endodermal sinus tumor diagnosed during pregnancy.

From the Departments of Gynecology,[a] Medical Oncology,[b] and Pathology,[c] University Hospital.
Received for publication September 25, 1989; accepted August 31, 1990.
Reprint requests: E.G.E. de Vries, MD, Division of Medical Oncology, Department of Internal Medicine, University Hospital, Oostersingel 59, Groningen, The Netherlands 9713 EZ.
6/1/25057

Case report

A 24-year-old woman in week 16 of her first pregnancy was screened for a neural tube defect of the fetus. The serum α-fetoprotein (AFP), measured with an enzyme-linked immunoassay (Abboth, Chicago), was elevated (22,000 ng/ml) (upper limit of normal at 16 weeks' gestation = 80 ng/ml). Serum human chorionic gonadotropin (hCG) level was normal.

Subfractionation of serum AFP, with concanavalin A crossed-line affinity immunoelectrophoresis, showed that 60% of the AFP was present in the nonbinding

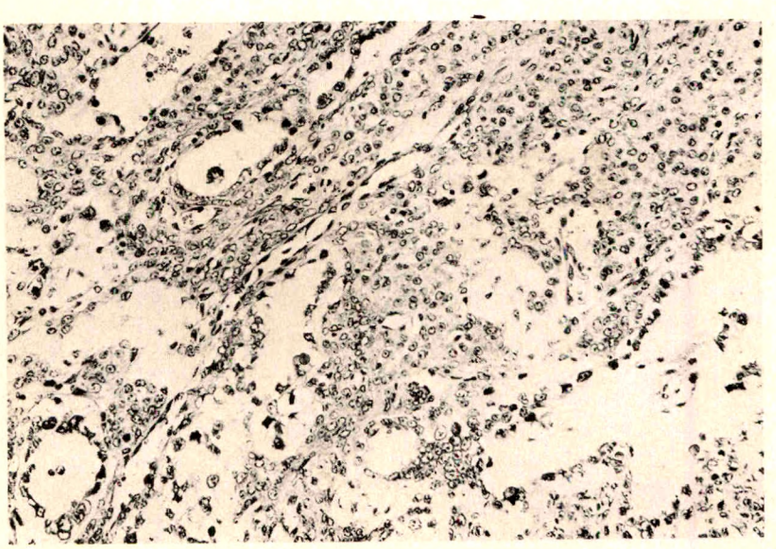


Fig. 1. Histology of endodermal sinus tumor, yolk sac tumor of ovary, with mixed histology including microcystic and alveolar glandular patterns (hematoxylin and eosin stain, × 140).

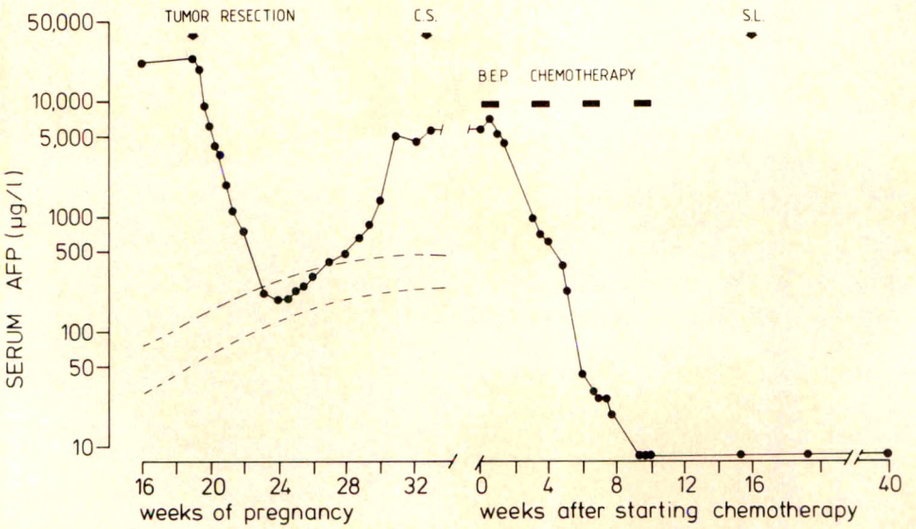


Fig. 2. Serum AFP profile during pregnancy and chemotherapy. *Dotted lines* represent median and 97th percentile as found during normal pregnancies. *Vertical arrows* indicate time at which laparotomies were performed. Courses of chemotherapy are indicated by *blocks*. *CS*, Cesarean section; *SL*, second look; *BEP*, cisplatin, etoposide, and bleomycin.

fraction. This is characteristic of AFP found in germ cell tumors.

At 18 weeks' gestation at rectovaginal examination a large pelvic mass was found. Ultrasonography showed a normal fetus and behind the uterus an irregular mass. The serum AFP level was now increased to 22,700 ng/ml. Laparotomy revealed a 10 × 12 cm tumor in the left ovary, adhesive in the cul de sac. The right ovary and the uterus were normal. No other tumor localizations were seen. Unilateral salpingo-oophorectomy was performed during which cystic fluid leakage occurred. A left pelvic lymphadenectomy and omentectomy were also performed.

Final diagnosis was endodermal sinus tumor (Fig. 1) without evidence of metastatic disease. The first four weekly assayed serum AFP levels fell to normal for the duration of the pregnancy, but increased from 28 weeks' gestation onward (Fig. 2). After 5 weeks, at 33 weeks' gestation, characterized by an acceptable perinatal mortality and morbidity for the fetus, a male infant with body weight of 1990 gm and Apgar score after 3 minutes of 9 was delivered by cesarian section. Biopsy specimens from multiple tumor nodules seen in the pelvis, the paracolic regions, and on the diaphragm showed endodermal sinus tumor. The infant was admitted to the neonatal intensive care unit because of hyaline membrane disease that was successfully treated. Further development was undisturbed. At 6 days after surgery, chemotherapy was administered intravenously with cisplatin 20 mg/m^2 days 1 to 5 with prehydration and posthydration; etoposide 120 mg/m^2 days 1, 3, and 5; and bleomycin 30 mg days 2, 8, and 16. This cycle of 21 days was repeated four times. Bleomycin was withheld in the fourth cycle because of pulmonary toxicity. After two cycles serum AFP levels were normal (Fig. 2). Second-look laparotomy was performed after the last course of chemotherapy. Examination of the multiple biopsy specimens showed no residual disease. The patient was disease free 24 months after chemotherapy. The serum AFP and serum hCG levels have remained normal.

Comment

Currently, most physicians agree that, even in the eary stage, surgical treatment alone of a patient with endodermal sinus tumor is no longer justified. The results of surgery followed by platinum-containing combination chemotherapy are good, yielding a 70% to 90% overall rate of long-term survivors. The results of combination chemotherapy with cisplatin, etoposide, and bleomycin are comparable to those obtained with cisplatin, vinblastine sulfate, and bleomycin, but with less long-term toxicity.[1] Therefore, we decided to treat this patient with the cisplatin, etoposide, and bleomycin regimen.

Most of the scarce cases found in the literature were reported before the era of combination chemotherapy. Malone et al.[2] successfully treated one patient with endodermal sinus tumor in week 20 of pregnancy with surgery and two courses of cisplatin, vinblastine, and bleomycin before delivery and three courses after delivery. Metz et al.[3] treated one patient with an endodermal sinus tumor during pregnancy with surgery and five courses of vincristine sulfate, doxorubicin hydro-

chloride, and Cytoxan preparation of cyclophosphamide from 17 weeks' gestation. After delivery was induced at 37 weeks she received an additional seven courses. The outcome was successful for the mother and the child. Similar case reports have not been published.

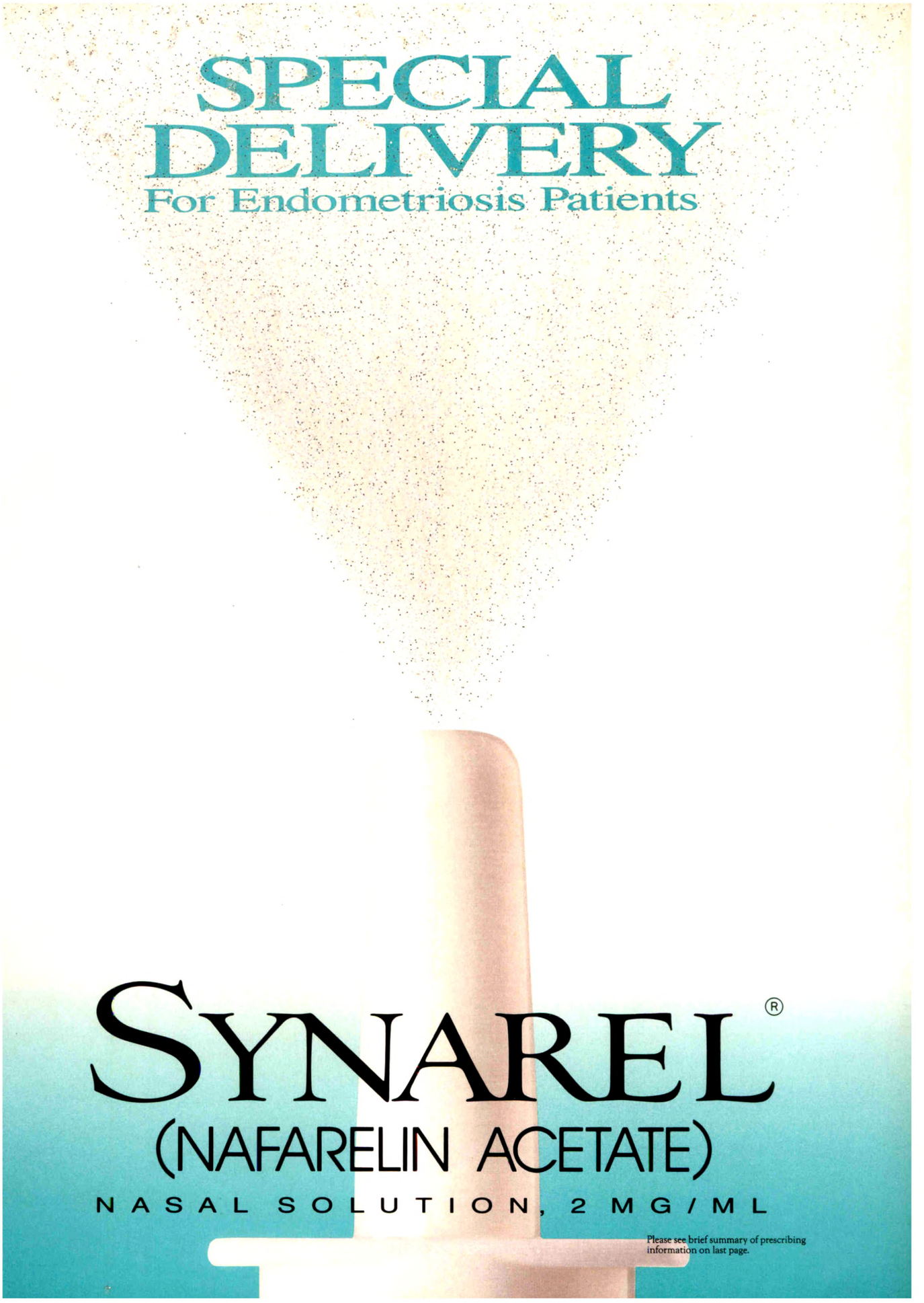
To our knowledge, no specific data exist on the long-term effects of the administration of cisplatin-containing combination chemotherapy during pregnancy with regard to the child. The incidence of congenital anomalies after other various combinations of chemotherapy in the second and third trimester of pregnancy is reported not to be higher than that in uncomplicated pregnancies. However, almost no information is available to determine the long-term risks of in utero exposure to chemotherapy. These risks include development and growth disturbances, gonadal disorders, immunosuppression, and genetic and teratogenetic disorders in future generations. Because of the potential risks of this type of chemotherapy and the well-known efficacy of cisplatin-containing regimens, we avoided chemotherapy during pregnancy.

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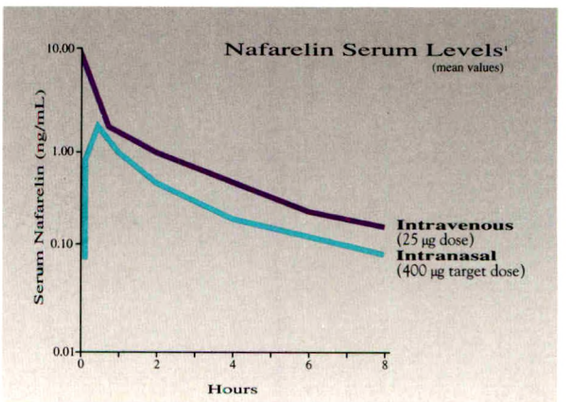
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INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.

CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.

WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.

When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.

PRECAUTIONS Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. Patients missing successive doses may experience breakthrough bleeding.

2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients.

3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician.

4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients.

5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content.

6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption.

7. Retreatment cannot be recommended since safety data beyond 6 months are not available.

Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.

Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.

Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.

Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.3% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (±SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 4.3% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

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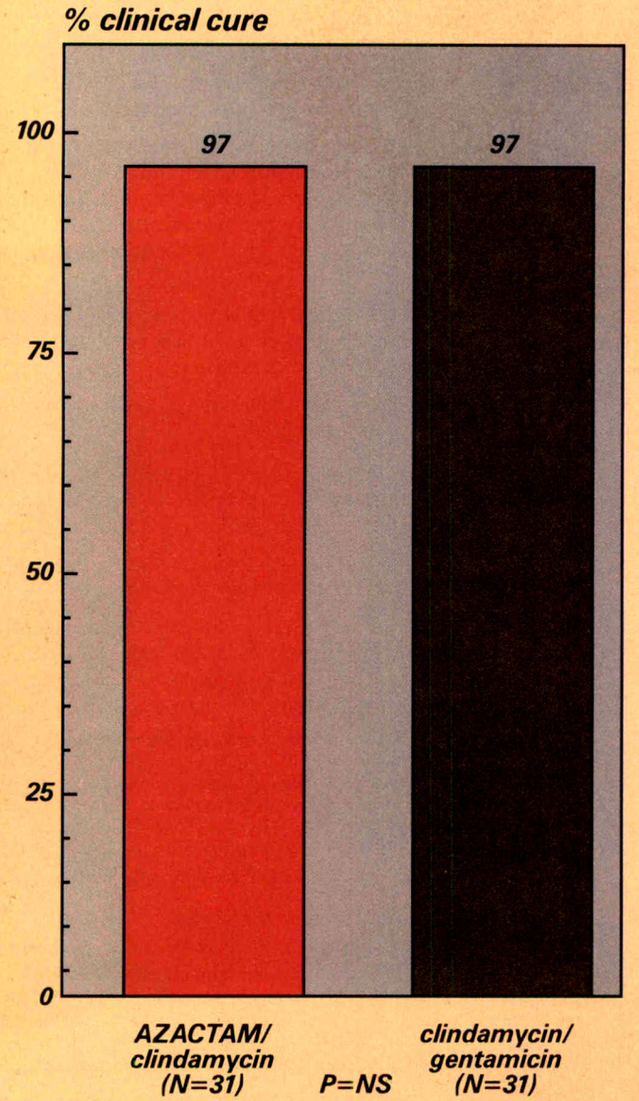
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A randomized, comparative trial of AZACTAM versus gentamicin, each with clindamycin, for the treatment of endometritis after delivery.[1]

No universal criteria exist that may satisfy a single definition of "cure." Use of this term may vary between clinical settings and investigators, and the reader is urged to consult the reference listed.

*Due to susceptible organisms.

†Although a useful guide, in vitro data do not necessarily correlate with therapeutic outcome.

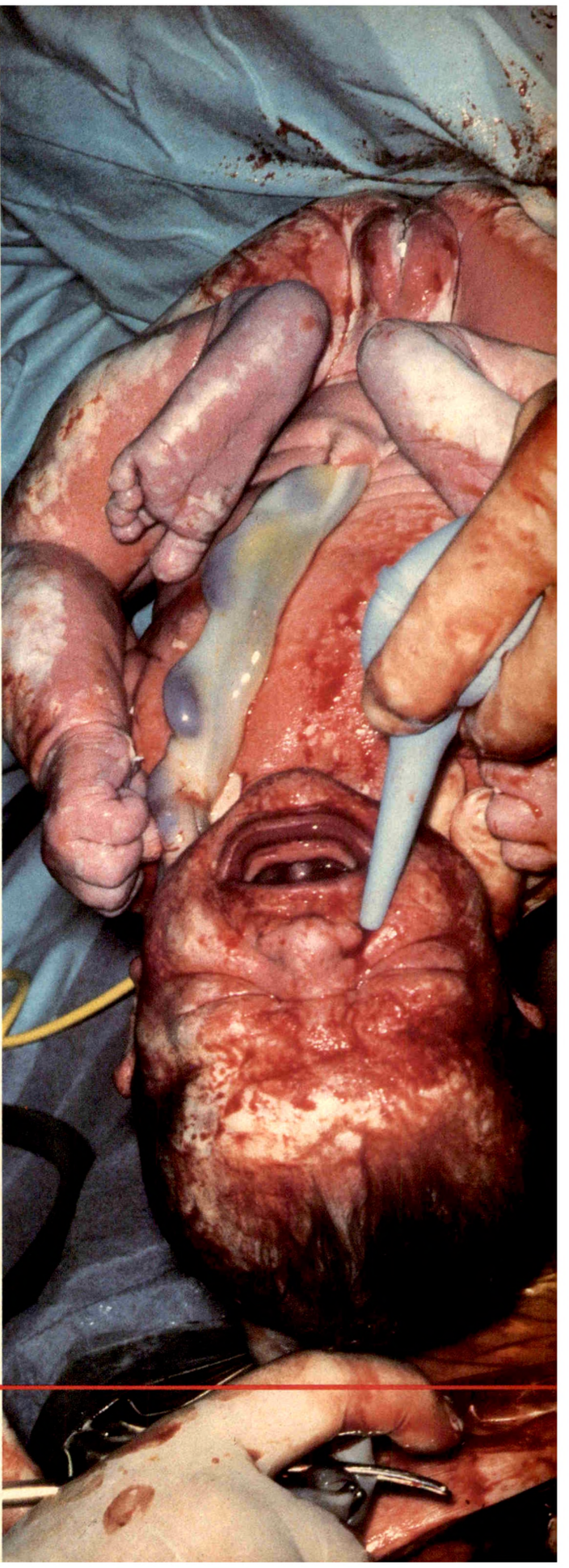
‡Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. See full prescribing information for recommendations on patients with immediate hypersensitivity reactions to penicillins.

Following IV administration, the most commonly seen adverse reactions occurred in less than 2% of patients, including local reactions, diarrhea, nausea/vomiting, and rash.

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- *Broad aerobic gram-negative in vitro coverage, including Escherichia coli, Proteus mirabilis, and Enterobacter cloacae.*†
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- *Pregnancy Category B.*

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DESCRIPTION–AZACTAM (aztreonam, Squibb) is the first member of a new class of antibiotics classified as monobactams. AZACTAM is a totally synthetic bactericidal antibiotic with activity against a wide spectrum of gram-negative aerobic pathogens. The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics.

AZACTAM For Injection is a sterile, nonpyrogenic, sodium-free, white to yellowish-white lyophilized cake, containing approximately 780 mg arginine per gram of aztreonam for intramuscular or intravenous use following constitution. Aqueous solutions of the product have a pH in the range of 4.5-7.5.

INDICATIONS AND USAGE–Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: **Urinary Tract Infections** (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca*, Citrobacter* species* and *Serratia marcescens**. **Lower Respiratory Tract Infections,** including pneumonia and bronchitis caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens**. **Septicemia** caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*, Serratia marcescens** and *Enterobacter* species. **Skin and Skin-Structure Infections,** including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Citrobacter* species*. **Intra-abdominal Infections,** including peritonitis caused by *Escherichia coli, Klebsiella* species, including *K. pneumoniae, Enterobacter* species including *E. cloacae*, Pseudomonas aeruginosa, Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**. **Gynecologic Infections,** including endometritis and pelvic cellulitis caused by *Escherichia coli, Klebsiella pneumoniae*, Enterobacter* species* including *E. cloacae**, and *Proteus mirabilis**.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy–Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organisms(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an antianaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS–Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS–Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin). Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

PRECAUTIONS–General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility–Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy–Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers–Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use–Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS–Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity; *Hypersensitivity*–anaphylaxis. *Hematologic*–pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. *Gastrointestinal*–abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). *Dermatologic*–purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. *Cardiovascular*–hypotension, transient ECG changes (ventricular bigeminy and PVC). *Respiratory*–one patient experienced flushing, chest pain, and dyspnea. *Hepatobiliary*–hepatitis, jaundice. *Nervous System*–seizure, confusion, vertigo, paresthesia, insomnia, dizziness. *Musculoskeletal*–muscular aches. *Special Senses*–tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. *Other*–vaginal candidiasis, vaginitis, breast tenderness. *Body as a Whole*–weakness, headache, fever, malaise.

Adverse Laboratory Changes–Those reported without regard to drug relationship during clinical trials were: *Hepatic*–elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*–increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Renal*–increases in serum creatinine.

OVERDOSAGE–If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION–Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg,** or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231C)

References: ***1.*** *Greenberg RN, Reilly PM, Weinandt WJ, et al: Comparison trial of clindamycin with aztreonam or gentamicin in the treatment of postpartum endometritis. Clin Ther 10(1):36-39, 1987.* ***2.*** *Gibbs RS, Blanco JD, Lipscomb KA, et al: Aztreonam versus gentamicin, each with clindamycin, in the treatment of endometritis. Obstet Gynecol 65:825-829, 1985.* ***3.*** *Henry SA: Overall clinical experience with aztreonam in the treatment of obstetric-gynecologic infections. Rev Infect Dis 7(suppl 4):S703-S708, 1985.*

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Endothelial-derived relaxing factor released by endothelial cells of human umbilical vessels and its impairment in pregnancy-induced hypertension

Aldo Pinto, PhD,[a] Raffaella Sorrentino, PhD,[a] Paola Sorrentino, MD,[b] Teresa Guerritore, MD,[b] Lea Miranda, PhD,[a] Augusto Biondi, MD,[a] and Pasquale Martinelli, MD[b]

Naples, Italy

The principal objective of this study was to determine and compare the capability of human umbilical vessels of normal and pregnancy-induced hyperensive parturients to produce and to release the endothelial-derived relaxing factor. A bolus of bradykinin injected in the perfusion system of human umbilical vessels induces a release of a relaxant factor, detectable by bioassay, that is pharmacologically similar to the endothelial-derived relaxing factor. Human umbilical cords were collected from normal and pregnancy-induced hypertensive parturients. In the latter group the release of endothelial-derived relaxing factor is extremely reduced. In fact, in umbilical vessels collected from normal parturients, bradykinin at a dose of 20 pmol produces a release of endothelial-derived relaxing factor equivalent to a relaxation induced by 58.9 ± 11.0 and 30.8 ± 11.4 pmol of glyceryl trinitrate for the artery and vein, respectively. The same dose of bradykinin in umbilical vessels, collected from pregnancy-induced hypertensive parturients, produces a release equivalent to 6.6 ± 2.2 and 5.7 ± 3.5 pmol of glyceryl trinitrate equivalent for the artery and vein, respectively. Neither an increasing bolus of exogenous bradykinin or an infusion of superoxide dismutase or L-arginine was able to restore the production of endothelial-derived relaxing factor to normal levels. Our results indicate a probable alteration of endothelial cell numbers or an alteration of the enzymatic pathway, probably due to cytotoxic endogenous factors produced in pregnancy. (AM J OBSTET GYNECOL 1991;164:507-13.)

Key words: Endothelial-derived relaxing factor, human umbilical vessels, pregnancy-induced hypertension

Endothelial cells release a labile relaxing substance, named endothelial-derived relaxing factor (EDRF), that also shows antithrombotic properties[1] in response to a wide number of vasoactive agents,[2] including acetylcholine or bradykinin. Recently it has been proposed that EDRF, or one of its components, could be nitric oxide[3] that has been formed by metabolism of L-arginine.[4] It now appears clear that EDRF is involved in the maintenance of blood pressure[5] and that hypertension induced genetically or experimentally or by an alimentary method is accompanied by a reduction of the production and/or release of EDRF.[6-8]

Approximately 10% of complications in pregnancy are due to some degree to pregnancy-induced hypertension. This hypertension can produce severe perinatal morbidity and mortality.[9] At present the causes of this hypertension have not been identified, although concomitant changes in a number of factors have been shown.[10-13] Recently, several reports have documented biochemical abnormalities in serum from patients with preeclampsia, which support the concept that endothelial cell perturbation and sublethal injury of these cells may contribute to the pathogenesis of pregnancy-induced hypertension. Most of these studies have shown elevated levels of endothelial cell products in serum (for example, fibronectin[10] or factor VIII antigen[11]), with changes in hemostasis, an increase in corticotropin-releasing factor,[12] and a modification of the kallikrein-kinin and prostaglandin systems.[13] At the moment there is no evidence for endothelial damage on the fetal side of pregnancies complicated by hypertension. The purpose of this study was to assess the production and release of EDRF by human umbilical cord vessels and to assess if there is any variation of its production and release in pregnancy-induced hypertension.

From the Department of Experimental Pharmacology[a] and the Institute of Gynecology, Obstetrics and Physiopathology of Human Reproduction, II Faculty of Medicine and Surgery,[b] University of Naples "Federico II."

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Reprint requests: Dr. Aldo Pinto, Department of Experimental Pharmacology, Via D. Montesano 49, 80131 Napoli, Italy.

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Methods and material

Experimental technique. The technique used was a modification of that developed by Gryglewski et al.[14]

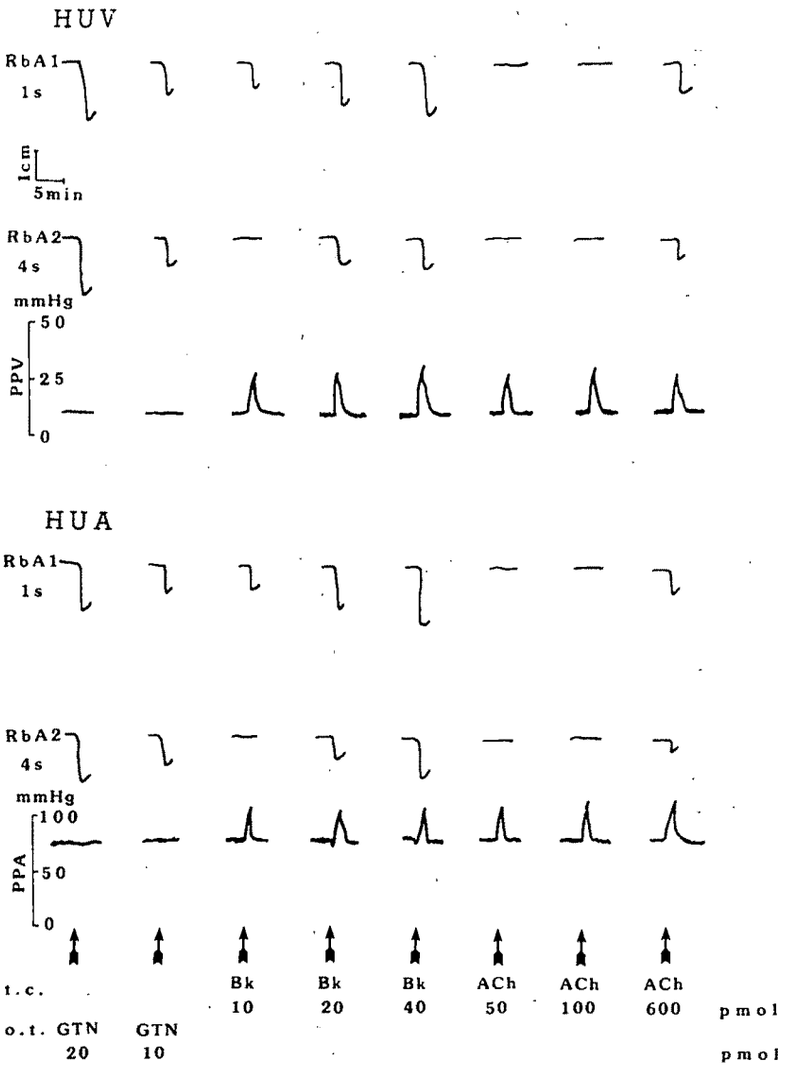


Fig. 1. Relaxation of strips of rabbit aorta by EDRF. The effluent of human umbilical vein *(HUV)* or artery *(HUA)*, from patients with normal pregnancy, perfused with Krebs buffer was used to superfuse two spiral strips of rabbit aorta which were separated from the umbilical vessel by delays of 1 second *(RbA1)* and 4 seconds *(RbA2)*, respectively. The tissues were contracted submaximally by a continuous infusion of phenylephrine (0.2 μmol/L). The sensitivity of the tissues was standardized with glyceryl trinitrate *(GTN)* administered over the tissues *(o.t.)*. The release of EDRF was stimulated by bolus injection through the cord *(t.c.)* of bradykinin *(Bk)* or acetylcholine *(ACh)*. Perfusion pressure for the artery *(PPA)* and the vein *(PPV)* was recorded.

and adapted to perfuse blood vessels.[15] The umbilical cords were collected from parturients after cesarean operation and immersed in Krebs bicarbonate solution with the following composition: sodium chloride, 118 mmol/L; potassium chloride, 4.7 mmol/L; calcium chloride, 2.5 mmol/L; magnesium sulfate, 1.2 mmol/L; potassium phosphate, 1.2 mmol/L; sodium bicarbonate, 25 mmol/L; glucose, 11.1 mmol/L. Ten-centimeter segments of human umbilical cord were taken and the umbilical vein or artery was cannulated at each end with polyethylene tubing and placed in a large organ bath containing gassed (95% oxygen plus 5% carbon dioxide) and warmed (37° C) Krebs solution. The vessel was perfused in the same direction of blood flow in vivo, with a flow rate of 2 ml/min of gassed Krebs solution at 37° C containing indomethacin (10 μmol/L). The perfusion in the artery started with a very low flow rate (0.250 ml/min) and gradually increased up to 2 ml/min when a steady state of perfusion pressure was reached to avoid a very high initial level of pressure. In the vein the perfusion started directly with a flow rate of 2 ml/min. The effluent from the

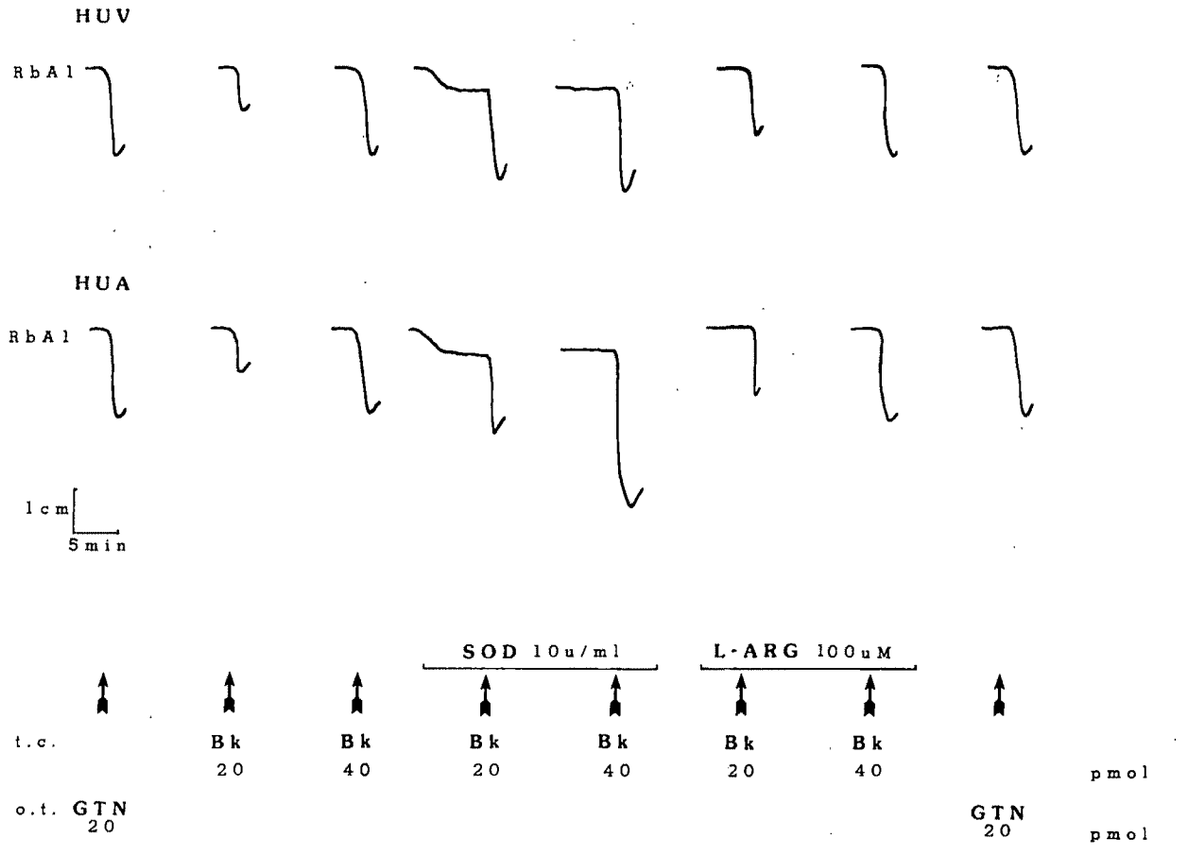


Fig. 2. Effect of superoxide dismutase *(SOD)* or L-arginine *(L-ARG)* on release of EDRF stimulated by bradykinin *(Bk)*. Effluent of human umbilical artery *(HUA)* or vein *(HUV)* of patients with normal pregnancy was used to superfuse spiral strip of rabbit aorta *(RbA1)* in a cascade. Glyceryl trinitrate *(GTN)* was used to test the reactivity of aorta strips.

vessel superfused a cascade of two spirally cut strips of rabbit thoracic aorta denuded of endothelium. The flow rate delay between the umbilical cord and consecutive first and second strips of rabbit thoracic aorta was 1 and 4 seconds, respectively. The bioassay tissues were contracted with a continuous infusion of phenylephrine hydrochloride (0.2 μmol/L over the tissues). Changes in the lengths of the bioassay tissues were monitored by isotonic transducers attached to a Gemini 7070 recorder (Basile, Lomerio, Italy). The sensitivity of the strips of rabbit thoracic aorta was adjusted so that they relaxed to a similar extent in response to a standard dose of glyceryl trinitrate. During all experiments the perfusion pressures of the umbilical vessels were monitored by a Statham pressure transducer. Human umbilical cords were collected after cesarean delivery of either normotensive pregnancy or pregnancy in which hypertension with proteinuria and edema (pregnancy-induced hypertension) occurred.

Patients. Patients were recruited from admissions to the Obstetrical Clinic of the Second Medical Faculty of Naples University. The diagnosis of pregnancy-induced hypertension was made on the basis of the criteria that systolic blood pressure was ≥140 mm Hg, diastolic pressure was ≥90 mm Hg, and proteinuria was ≥30 mg/dl with diffused edema to the limbs. The normotensive group contained patients who underwent cesarean delivery and had no alteration of blood pressure or any proteinuria or edema. None of the patients had a history of hypertension or renal disease before pregnancy. Subjects within the normotensive group did not undergo drug administration during pregnancy whereas the pregnancy-induced hypertension group were treated with antihypertensive agents, i.e., diuretics, methyldopa, β-adrenergic receptor antagonist or α_1-adrenergic receptor antagonists, but not with calcium channel blockers. The cesarean surgery was done at term pregnancy for the normotensive group and at 36.5 ± 1.5 weeks of gestation for the pregnancy-induced hypertension group.

Material. Phenylephrine hydrochloride, acetylcholine chloride, bradykinin, hemoglobin, L-arginine chloride, and superoxide dismutase were purchased from Sigma Chemical Co. (St. Louis). Glyceryl trinitrate (Ni-

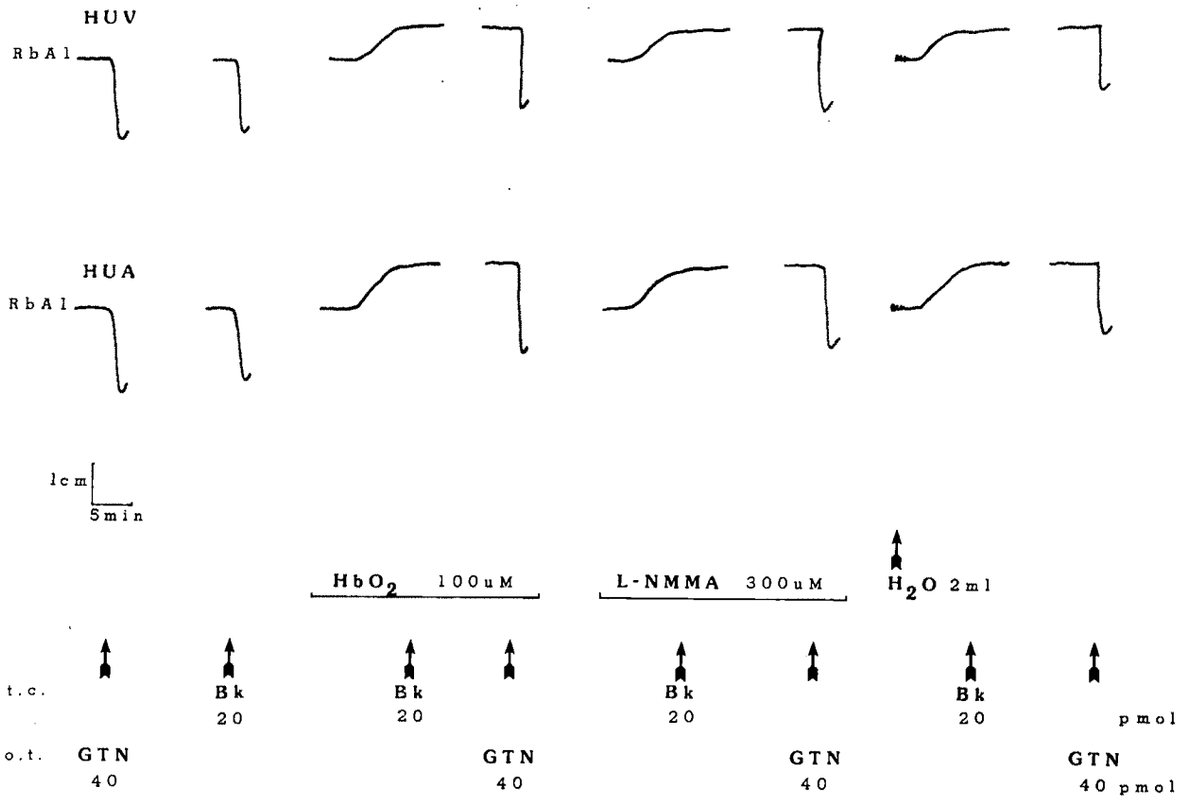


Fig. 3. Inhibitory effect of oxyhemoglobin *(HbO₂)* or L-N^G-monomethylarginine *(L-NMMA)* and destruction of endothelial cells by a flash of distilled water *(H_2O)* on release of EDRF stimulated by bradykinin *(Bk)*. Effluent of human umbilical artery *(HUA)* or vein *(HUV)* of patients with normal pregnancy was used to superfuse a rabbit aorta spiral strip *(RbA1)* in a cascade. Glyceryl trinitrate *(GTN)* was used to test the rabbit aorta strip reactivity.

tronal) was purchased from Lipha Pharmaceuticals Ltd. (Middlesex, United Kingdom). L-N^G-monomethyl arginine (Italfarmaco, Milan, Italy) was a gift from Dr. G. De Nucci. Oxyhemoglobin was prepared as described by Buga et al.[16]

Statistics. The amount of EDRF released was estimated in terms of relaxation of the bioassay tissues with glyceryl trinitrate used as standard, and results were expressed as picomoles of glyceryl trinitrate equivalent. Statistical analysis was performed with the two-way repeated measures analysis of variance test (two-tailed analysis), and a value of $p < 0.05$ was taken to be significant.

Results

Normotensive group. Bolus injection of acetylcholine or bradykinin through the umbilical artery or vein, but not over the tissues, produced release of a factor that was able to relax the precontracted strips of rabbit thoracic aorta (Fig. 1). Bradykinin was the more active substance in causing release of the relaxing factor from rabbit thoracic aorta strips, as compared with acetylcholine ($p = 0.001$). In fact, bradykinin in a dose range of 5 to 40 pmol ($n = 7$) produced a relaxation of rabbit thoracic aorta strip 1 equivalent to 10.7 ± 3.4 to 85.9 ± 7.9 pmol of glyceryl trinitrate, whereas only the highest dose of acetylcholine (600 pmol; $n = 5$) produced a small relaxation equivalent to 14.5 ± 4.5 pmol of glyceryl trinitrate. Between two administrations of acetylcholine or bradykinin we had to wait at least 30 minutes to have a repeated reproducible response. Since bradykinin was more active and reproducible, it was used in all the following experiments to characterize the nature of the factor.

To identify this bradykinin-induced relaxing factor as EDRF, we performed suitable experiments that produced these results:

1. The relaxation observed on rabbit thoracic aorta strip 1 declined during passage down the cascade on rabbit thoracic aorta strip 2 (Fig. 1). In contrast, the relaxation caused by glyceryl trinitrate did not decline (Figs. 1 and 2).

2. Infusion of superoxide dismutase (10 U/ml) or L-arginine (100 μmol/L) enhanced the release and/or production of the relaxing factor from both the vein and artery by about 3.5 times (Fig. 2). During superoxide dismutase infusion a decrease of basal tone induced by phenylephrine was observed.

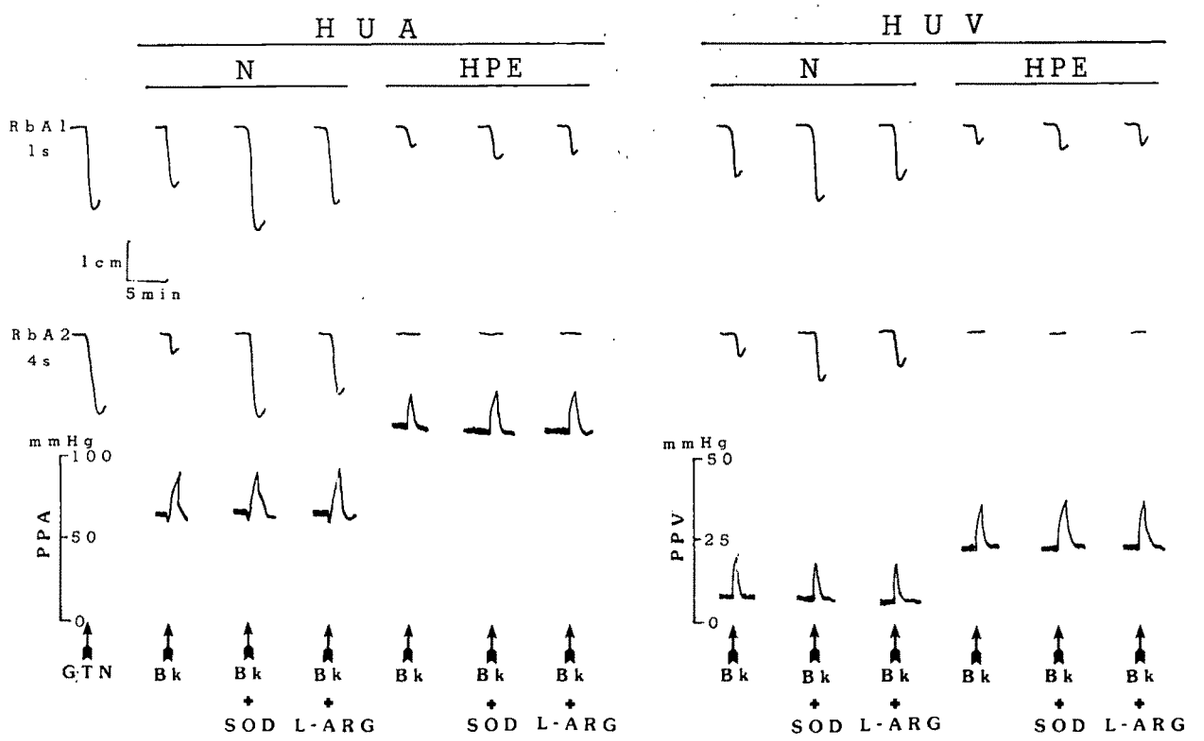


Fig. 4. Comparison between EDRF released from endothelial cells of human umbilical artery *(HUA)* or vein *(HUV)* of patients with normal pregnancy *(N)* and pregnancy-induced hypertension *(HPE)*. Bradykinin *(Bk)* was injected through the cord vessels in a bolus (20 pmol). There was a drastic reduction of EDRF production, and infusion of superoxide dismutase *(SOD,* 10 U/ml) or L-arginine *(L-ARG,* 100 μmol/L) was not able to restore normal production of EDRF in the *HPE* group. The reduction of EDRF released from *HUA* or *HUV* among the *HPE* group is associated with an increase of perfusion pressure of artery *(PPA)* and vein *(PPV)*. Glyceryl trinitrate, over the tissues, was used to test the reactivity of rabbit aorta strips (*RbA1* and *RbA2*) in cascade.

3. By destroying the endothelial cell monolayer with a bolus injection of distilled water (2 ml) or an infusion of oxyhemoglobin (100 μmol/L) or of L-N^G-monomethyl arginine (300 μmol/L), two inhibitors of EDRF formation, we observed a total disappearance of the relaxation produced by 20 pmol of bradykinin (Fig. 3). The flash of distilled water did not affect the smooth muscle functions of strips of rabbit thoracic aorta as shown by glyceryl trinitrate reactivity of the strips (Fig. 3) and the smooth muscle functions of the umbilical vessels as demonstrated by a sharp bradykinin-induced increase of perfusion pressure (data not shown). In addition, after a water flash or an infusion of inhibitors of EDRF production, there was an increase of basal tone of the strips of rabbit thoracic aorta by about 10% (Fig. 3). For the normotensive group the perfusion pressure in the artery, with an initial flow rate of 0.250 ml/min, started with 33.8 ± 9.9 mm Hg and declined to a steady, stable value of 12.5 ± 3.4 mm Hg in 7 to 10 minutes; then the flow rate was increased gradually by 0.250 ml/min at a time until a rate of 2 ml/min was reached and produced a steady, stable value of 71.4 ± 6.9 mm Hg. In the vein the flow rate of 2 ml/min produced a perfusion pressure of 50.3 ± 12.4 mm Hg that declined to a final pressure of 8.1 ± 1.3 mm Hg in 10 to 15 minutes. Bolus injection of bradykinin in the normotensive group produced a slight reduction of perfusion pressure immediately followed by a sharp, non-dose-dependent increase of pressure in the umbilical artery (Fig. 1). In the vein, in the same group, only the sharp, non-dose-dependent increase of pressure was observed.

Pregnancy-induced hypertension group. In the pregnancy-induced hypertension group bradykinin-induced EDRF release was reduced, as compared with that of the control (normotensive group) by 89.0% ± 10.4% (n = 5) in the artery and 81.4% ± 15.1% (n = 4) in the vein (Fig. 4 and Table I). In this group an infusion of superoxide dismutase or L-arginine produced an increase in the release and/or detection of EDRF but was not able to restore the amount detected to control levels (Fig. 4 and Table I).

For the pregnancy-induced hypertension group the perfusion pressure in the artery, with an initial flow rate of 0.250 ml/min, started with 65.2 ± 17.8 mm Hg that declined to a steady, stable value of 20.9 ± 7.5

Table I. EDRF expressed as picomoles of glyceryl trinitrate equivalent released by endothelium of human umbilical vessels from normal pregnancy and pregnancy-induced hypertension

Drug	*Dose*	*Artery*		*Vein*	
		Normal pregnancy	*Pregnancy-induced hypertension*	*Normal pregnancy*	*Pregnancy-induced hypertension*
Bradykinin	20 pmol	59.9 ± 11.0 (n = 7)	6.6 ± 2.2* (n = 5)	30.8 ± 11.4 (n = 7)	5.7 ± 3.5† (n = 4)
Bradykinin plus superoxide dismutase	20 pmol plus 10 U/ml	200.9 ± 54.6 (n = 6)	24.7 ± 9.7* (n = 4)	115.8 ± 37.1 (n = 6)	15.0 ± 6.2† (n = 4)
Bradykinin plus L-Arginine	20 pmol plus 100 μmol/L	114.8 ± 16.7 (n = 5)	15.8 ± 2.6* (n = 4)	44.5 ± 16.4 (n = 5)	13.1 ± 6.9† (n = 3)

Results are expressed as mean ± SEM.
*$p < 0.005$, pregnancy-induced hypertension versus normal pregnancy.
†$p < 0.05$, pregnancy-induced hypertension versus normal pregnancy.

Table II. Values of perfusion pressure for artery and vein of human umbilical cord of normal pregnancy and pregnancy-induced hypertension

Perfusion pressure	*Normal pregnancy*	*Pregnancy-induced hypertension*
Artery (mm Hg)	71.4 ± 6.9 (n = 9)	123.8 ± 30.5* (n = 6)
Vein (mm Hg)	8.1 ± 1.3 (n = 7)	21.6 ± 5.0* (n = 6)

Results are expressed as mean ± SEM.
*$p < 0.005$.

mm Hg in 10 to 15 minutes. Then the flow rate was increased gradually by 0.250 ml/min at a time until a rate of 2 ml/min was reached and produced a steady, stable value of 123.8 ± 30.5 mm Hg. In the vein the flow rate of 2 ml/min produced a perfusion pressure of 85.4 ± 15.7 mm Hg that declined to 21.6 ± 5.0 mm Hg in 10 to 15 minutes (Table II). Three umbilical cords from the pregnancy-induced hypertension group were discarded because they showed an initial perfusion pressure over 200 mm Hg. Also in umbilical vessels from pregnancy-induced hypertension group, the injection of a bradykinin bolus of 20 pmol produced a sharp, non-dose-dependent increase of perfusion pressure. However, the light reduction of perfusion pressure observed in the artery of the normotensive group, disappeared in the artery of the pregnancy-induced hypertension group.

Comment

Vascular endothelium generates an unstable vasodilator named EDRF.[2] Because of the lack of stability of EDRF in its physiologic environment (a half-life of about 6 seconds[14]), it can only be detected by biologic methods.

Umbilical vascular endothelium also generates prostacyclin and prostaglandin E_2.[15] The bioassay of EDRF is not influenced by the presence of prostacyclin or prostaglandin E_2 since neither of these two prostanoids affects the tone of the strips of rabbit thoracic aorta and the presence of indomethacin precludes the formation of prostanoids.

Bradykinin and acetylcholine are well characterized as inducers of EDRF release from almost all vascular endothelium. In this system, which uses the vessels of the human umbilical cord, the EDRF released in response to bradykinin and acetylcholine relaxed precontracted strips of rabbit thoracic aorta in a manner pharmacologically identical to that described for EDRF by several authors in mammals[2] and in human umbilical vessels.[16, 17] The relaxation is abolished after destruction of endothelial cells by a flash of distilled water and inhibited by oxyhemoglobin or L-N^G-monomethyl arginine.[4] Conversely, its detection increased by infusion of superoxide dismutase[18] or L-arginine.[4] Infusion of oxyhemoglobin or L-N^G-monomethyl arginine or a flash of water caused an increase in the contraction of the strips of rabbit thoracic aorta indicating a basal release of EDRF from the endothelium of human umbilical cord vessels, which was also confirmed by a decrease of the basal tone of strips of rabbit thoracic aorta observed with superoxide dismutase infusion. A further proof of the EDRF basal release was obtained with a double perfusion system: a direct superfusion line and a through-umbilical-vessel superfusion line. We observed a decrease of the basal tone of strips of rabbit thoracic aorta with the switch from a direct line to the umbilical vessel line (data not shown).

Bradykinin was more active than acetylcholine in releasing EDRF from human umbilical cord vessels, as was also observed by other authors.[17] This is possibly due to differences in the number of receptors present on the endothelium. It is interesting that there is an increase of plasmatic kininogen and urinary kallikrein in pregnancy and that infusion of bradykinin[13] or administration of kininase II inhibitor increases uterine blood flow.[19] Thus there is evidence to suggest a role

for bradykinin in the control of vascular tone and placental blood flow in pregnancy. This is further supported by experiments demonstrating that urinary kallikrein excretion falls to levels beneath those of a normal nonpregnant individual in patients in whom hypertension develops during pregnancy.[13]

A decrease in EDRF release has been observed in vitro in vessels of spontaneously[6] or experimentally[7] hypertensive animals, although the precise mechanism has not been established. In the anesthetized rabbit L-N^G-monomethyl arginine induces a dose-dependent, long-lasting increase in mean arterial blood pressure,[5] suggesting that a reduction in the synthesis of EDRF may contribute to the genesis of hypertension. These results are supported by our study, as there is a drastic reduction of bradykinin-induced EDRF release from the endothelium of human umbilical cord vessels collected from patients with pregnancy-induced hypertension and an increase in the perfusion pressure in both the artery and the vein. This could well be due to a reduction of the basal release of EDRF, also confirmed by the lack of reduction of the basal tone of strips of rabbit thoracic aorta with infusion of superoxide dismutase.

This reduction of EDRF production is not due to the decrease of circulating bradykinin or substrate (L-arginine) or to an increase of superoxide anions, because addition of exogenous bradykinin, L-arginine, or superoxide dismutase did not restore the detection of EDRF to normal levels. Possibly, then, there may be changes in the number of endothelial cells or in the enzymatic pathway of EDRF production within endothelial cells during pregnancy-induced hypertension.

The cause(s) of pregnancy-induced hypertension, the specific disease of pregnancy, is not yet clear because too many changes are involved in this pathologic condition (see Sullivan[13]). It is possible that the reduction of EDRF release from the umbilical vessels, which are structurally part of the fetal circulation, could reflect a decrease in EDRF release throughout the maternal circulation. The results of this study support such a hypothesis and are consistent with recent findings of a direct cytotoxic effect of serum from preeclampsia on cultured human endothelial cells of the umbilical vein, an effect that was markedly diminished post partum.[20, 21]

In conclusion, the presence of elevated serum endothelial cytotoxins and mitogens in prepartum sera from patients with preeclampsia could be consistent with these results of a reduction of EDRF release from the endothelium of umbilical vessels in pregnancy-induced hypertension and could support the hypothesis that endothelial injury is a major pathogenic factor in preeclampsia.

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Prenatal records: A national survey of content

Mary D. Peoples-Sheps, DrPH, William D. Kalsbeek, PhD, Earl Siegel, MD, MPH, Cornelia Dewees, CNM, MN, Mary Rogers, MSN, and Robert Schwartz, MA
Chapel Hill, North Carolina

The uses of prenatal records extend well beyond management of patient care. They are also vehicles for communication, quality assurance, financial compensation, and legal documentation. To serve each of these functions, records should be systematic and detailed. This nationwide study was conducted to assess the extent to which prenatal records in current use include sufficient detail to serve these functions. A random sample of 2746 physicians who listed obstetrics and gynecology, obstetrics, or maternal-fetal medicine as a primary or secondary specialty in the American Medical Association file were contacted by mail and were requested to submit a blank copy of their prenatal records. The records of 969 respondents were examined for the presence or absence of 53 items that corresponded to the five functions identified above. The items of interest were present in 1% to 98% of the records. More than 90% included items of traditional medical-obstetric significance. The percentages of records with items of more contemporary relevance (e.g., cigarette smoking, risk-assessment checklists, psychological stress) were found in lower percentages of the records. Stratified and logistic regression analyses revealed that the most systematic and detailed records were commercially available, were used by physicians in hospital and government program-based practices, and were used by physicians who had completed medical school ≤15 years before the survey. The findings suggest that physicians who do not use commercially available forms or those who are in private or health maintenance organization practice or have been practicing for more than 15 years should reconsider their prenatal records in light of the functions they will serve in the 1990s and beyond. (Am J Obstet Gynecol 1991;164:514-21.)

Key words: Medical records, prenatal records, quality of medical records, uses of medical records

The demands placed on prenatal records in recent years are extensive and are becoming increasingly complex. In the past, these records were used primarily for the management of patient care, but today, prenatal records serve four additional major functions: communication, quality assurance, compensation, and documentation for legal purposes. Detailed information is required to perform these functions, although as Table I shows, different sets of information are required for each function.

Management of patient care. Prenatal records are considered major tools in the management of patient care. To serve this function effectively, they should include information in each of the nine categories shown in Table I.[1-3] Because there are many specific items that can be included in each category, records with detailed systematic formats have been developed. These formats are believed to enhance the content of care by prompting providers to include elements of prenatal surveillance and intervention that might otherwise be omitted.

Communication. Effective communication among maternity care providers and among service facilities is essential to the appropriate management of patient care. The prenatal record is the major vehicle for this communication, and all the nine types of information shown in Table I are required to serve this function. Because prenatal records are the primary tools for transmitting information to providers in inpatient facilities, it is especially important that they include space to document the involvement of other elements of the health care system, such as hospitalization during the prenatal period and referral to other health care providers. This information provides a comprehensive picture of the care received.

Quality assurance. Prenatal medical records are also important in monitoring compliance with standards for patient care. The records are particularly useful for this purpose if patients' problems, the processes of care, and corresponding results are systematically documented. The Professional Standards Review Organizations and other peer review and quality assurance groups rely on medical records to assess these param-

From the Department of Maternal and Child Health, School of Public Health, University of North Carolina.
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Reprint requests: Mary D. Peoples-Sheps, DrPH, School of Public Health, University of North Carolina, Rosenau Hall, CB 7400, Chapel Hill, NC 27599-7400.
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Table I. Type of information usually required for each major function of prenatal records

Type of information	*Record function*				
	Patient care managment	*Communication*	*Quality assurance*	*Compensation*	*Documentation for legal purposes*
Background data, especially socioeconomic	x	x	—	x	—
Patient history including medical, family, obstetric, behavioral	x	x	x	—	x
Initial, serial pregnancy surveillance	x	x	x	x	x
Patient education, special referrals	x	x	—	—	—
Complications developing problems	x	x	x	x	x
List of risks in present pregnancy	x	x	x	—	x
Laboratory tests	x	x	x	x	x
Other diagnostic procedures	x	x	x	x	x
Therapeutic procedures	x	x	x	x	x

eters.[2-4] For example, screening for sexually transmitted diseases during pregnancy is a standard of care that can be evaluated from information in this record. The reviewer should be able to determine whether testing was performed, the results of testing, whether patients with positive tests were identified and treated, and whether a test of cure was performed. Record formats that include space for the documentation of repeated laboratory testing and for medical treatment encourage recording the necessary information.

Compensation for services. Prenatal records provide documentation for out-of-pocket and third-party billing. This function is becoming increasingly important, inasmuch as Medicaid and some other insurers are "unbundling" maternity services. Under this framework, services are reimbursed by visit according to the content and complexity of care, rather than at a standard fee for a maternity care package. Thus accurate and complete documentation of patient complications and of services provided is essential for full reimbursement.

Documentation for legal purposes. The traditional functions served by prenatal records have been overshadowed in recent years by medicolegal functions. Appropriate documentation of health problems and interventions is an essential component of a provider's response to an obstetric malpractice suit. The frequency of these lawsuits has increased, and the need for clear documentation to show that adequate care was given has become more compelling. As shown in Table I, the types of information required for documentation for legal purposes are the same as those required for quality assurance. The record should show that risk factors and complications were properly identified, and that the course of pregnancy was managed in an appropriate manner.[5]

To be useful, prenatal records should be problem-oriented, systematic, and sufficiently detailed. Some formats, such as checklists for risk assessment and flow charts to detail the results of each physical assessment, have been developed to make information easily available. At least two problem-oriented maternity record systems are commercially available.[6,7] Other record systems have been developed for internal use by private providers and agencies,[8,9] and the American College of Obstetricians and Gynecologists has developed a brief prenatal record that became available late in 1989.[10]

The availability of systematic prenatal records and the important patient care, communication, quality assurance, compensation, and legal rationales for using systematic records argue in favor of widespread use. Until this study, however, actual levels of use were unknown. The purpose of this study is to assess the extent to which systematic and detailed maternity records are used by providers of prenatal care.

Material and methods

The sample for the study consisted of 2746 physicians who had been selected at random from a master file maintained for the American Medical Association. The file is considered virtually complete.[11] In this article, the term "physician" is used for both medical doctors and for doctors of osteopathy. The original sample of 2746 included 2656 medical doctors and 90 doctors of osteopathy. The sample of 969 analyzed in this article included 940 medical doctors and 29 doctors of osteopathy. Only physicians who were practicing at the time of the study and who listed obstetrics and gynecology, obstetrics, or maternal-fetal medicine as a primary or secondary specialty were eligible for inclusion. Systematic sampling after a random start from a list ordered by the zip code for the address of the physician's practice was used as the selection method.

A survey packet that included a cover letter, a brief questionnaire, and a prepaid business reply envelope was mailed to each selected physician. The cover letter explained the purpose of the study and requested that

Table II. Physician response rates by primary specialty and geographic region

		Response (%)		
	No.	*Questionnaire only (n = 512)*	*Questionnaire and record (n = 969)*	*No response (n = 1265)*
Primary specialty				
OB-GYN, GYN, MFM	2211	16.4	38.0	45.6
Other	535	27.9	24.1	48.0
Geographic region*				
Northeast	658	19.0	27.7	53.3
South and central	1107	21.1	34.0	44.9
West	981	15.6	41.9	42.5
TOTALS	2746	18.6	35.3	46.1

OB-GYN, Obstetrics and gynecology; *GYN,* gynecology; *MFM,* maternal-fetal medicine.

*Regions were derived from the first digit of zipcodes as follows: 0,1 = northeast; 2,3,4,6 = south and central; 5,7,8,9 = west.

Table III. Selected characteristics of 969 respondents, national prenatal records survey

Characteristics	%
Primary specialty	
OB/GYN, OB, MFN	87.7
Other	12.3
Type of practice	
Private solo	46.0
Private group	43.1
HMO	4.8
Other (i.e., hospital-based and government programs)	6.2
Time since graduation (yr)	
≤ 15	40.1
> 15	59.9
Region by first digit of zip code*	
0	7.9
1	10.8
2	9.3
3	11.0
4	10.5
5	5.5
6	7.9
7	11.1
8	6.0
9	19.8
Type of prenatal record submitted	
Hollister	13.5
POPRAS	2.3
Hospital-developed	33.8
Health department or government program-developed	2.3
Other†	48.1

OB-GYN, Obstetrics and gynecology; *OB,* obstetrics; *MFN,* maternal-fetal medicine.

*The states covered by the first digit of each zip code are 0: Massachusetts, Rhode Island, New Hampshire, Vermont, Maine, Connecticut, New Jersey; 1: New York, Pennsylvania; 2: Washington, D.C., Maryland, Virginia, West Virginia, North Carolina, South Carolina; 3: Georgia, Florida, Alabama, Tennessee, Mississippi; 4: Kentucky, Ohio, Indiana, Michigan; 5: Iowa, Wisconsin, Minnesota, South Dakota, North Dakota, Montana; 6: Illinois, Missouri, Kansas, Nebraska; 7: Louisiana, Arkansas, Oklahoma, Texas; 8: Colorado, Wyoming, Idaho, Utah, Arizona, New Mexico, Nevada; 9: California, Hawaii, Oregon, Washington, Alaska.

†Usually practice-specific.

respondents send us a blank copy of one complete prenatal record and the completed questionnaire in the prepaid envelope. The questionnaires were designed to obtain some basic practice and demographic data from respondents.

A pretest was conducted on 10% (276) of the sample in March 1987. The instruments and procedures were then revised slightly, and the main survey was started in May 1987. A reminder letter was sent 3 weeks after the initial mailing to those who had not responded.

Response rates for the survey are shown in Table II. A total of 54.4% of physicians responded and blank prenatal records were submitted by 38.0%. Table II also shows the distribution of physician responses by primary specialty and geographic region as defined by the first digit of the zip code for the physician's mailing address. Response rates differed across groupings; for this reason, weights were created to adjust for the variation.

Each record was reviewed for the presence or absence of 53 specific items or groups of items. These items were selected because they represent each of the essential types of record information listed in Table I. Efforts were made to select a set of items that showed a balance between traditional and more contemporary relevance to prenatal care. Hence, emphasis was given to items such as patient education, smoking, and other behaviors, as well as to more traditional medical and obstetric items. For an item to be coded "present," an unambiguous label for that item had to be in the record. A space to write conditions or procedures without the specified label was not sufficient for a code of "present."

From the original set of 53 items and indictors (i.e., groups of items), we selected 10 for in-depth analysis. These items were present in 11% to 89% of the records and were considered a representative subset of the original 53. They are the dependent variables defined in the appendix.

The appendix also includes definitions of five independent variables that were used in both stratified and logistic regression analysis. Three of the variables (primary specialty, type of practice, and number of years since graduation from medical school) were collected from respondents on the survey questionnaire. Location of the practice was determined from the first digit of the zip code. Type of prenatal record was ascertained from the record itself.

Cutoff points for each of these dichotomous independent variables were based on hypothetic grounds because there has been little research in this area. The cutoff points for two of the variables (type of practice and location) may not be intuitively obvious. Initially, health maintenance organization (HMO) practices (n = 47) were grouped with hospital and government program-based practices. However, preliminary analyses suggested that the presence of items in records of HMO physicians was more consistent with those of private physicians than those of hospital or government program-based physicians. Thus HMOs were grouped with private practices. With regard to location, the decision to include the Northeast and California in one group and the rest of the country in the other was based on the hypothesis that areas with numerous prenatal care services and large medical complexes would be more likely to have comprehensive prenatal records, primarily as a result of more rapid dissemination of new information, peer pressure, and geographic ease of oversight.

All reported findings from the survey reflect the design and weighting adjustments that were made to compensate for differential nonresponse. The data were adjusted by a weighting class procedure that used primary specialty and geographic region to define the adjustment cells.[12] Two statistical programs were used. The standard errors program for computing of standardized rates from sample survey data (SESUDAAN)[13] computer software package enabled us to estimate the percentage of records with items present, and a logistic regression (RTILOGIT)[14] was used to estimate coefficients for the assumed logistic regression program for analysis of survey data models used to identify predictors of the presence of certain items in the records.

Results

In Table III, distributions of selected characteristics of the 969 respondents who submitted prenatal records are presented. Almost 90% of the respondents specialized in obstetrics and gynecology, obstetrics, or maternal-fetal medicine, and a similar percentage was in either solo private practice or group private practice. A total of 40% had completed basic professional training 15 or fewer years before the survey. With regard to geographic region, there was considerable variation, which ranged from 5.5% in areas with zip codes starting with 5 to 19.8% where 9 was the first digit of the zip code. Finally, almost half of these respondents submitted a prenatal record that was not developed by one of the commercial producers [Problem-oriented perinatal risk assessment system (POPRAS) or Hollister] or by a hospital, health department, or government program.

Table IV contains estimates of the percentage of physicians' records with the 53 prenatal care items or item groups described earlier. The half-widths of 95% confidence intervals reported parenthetically indicate (in percentage points) the likely error due to sampling in these estimates. Percentage estimates range from 1% to 98%. The items that were present in >90% of the records were of traditional medical-obstetric significance, including such items as date of last menstrual period, description of previous pregnancies, and flow chart with standard items. At the opposite end of the spectrum are many items that have been recognized as relevant only in recent years. Indicators of psychological and financial stress, of a change in consumption of cigarettes, and of chlamydia test results, for example, were found in <10% of the records. However, it is important to note that even some widely accepted prenatal practices did not have clearly identifiable labels. A space to record the indication for ultrasonography was present in only 10% of the records, and 4% included an entry for prenatal hospitalizations.

Table V shows weighted estimates of the percentages of prenatal records with the 10 items selected for further analysis (dependent variables) by five of the characteristics of respondents (independent variables). Several trends are apparent in Table V.

All the POPRAS and Hollister records included eight of the items. The smoking and drinking items were present in 89% of those records because POPRAS records did not include them until the 1985 revision. In striking contrast to POPRAS and Hollister, none of the items was in >60% of records developed by others. Items found in <20% of the records developed by others were the quantity of cigarettes smoked and the checklist items such as entries for intrauterine growth retardation, sexually transmitted diseases, and for assigning prenatal risk status. Spaces to document general patient education, nutritional assessment, and demographics were available in 20% to 30% of the records of the other group.

In Table V, no percentages of other independent variables were smaller than 22 or greater than 80, and differences between categories tended to be smaller than in the type of record comparison. Differences in percentages by primary specialty and geographic region tended to be quite small.

More variation was found across categories of type of practice and number of years since graduation from

Table IV. Weighted estimates of the percentage of prenatal records with each item or indicator of interest

Item or indicator	*% Present*	*95% Half-width**
Background data		
Basic minimum sociodemographic data (age, race, education, marital status)	36	±3
Patient history		
Medical history	96	±2
Family history		
Congenital anomolies or birth defects	66	±3
Information about the nature of the birth defect	59	±3
Diabetes mellitus	7[illegible]	±3
Obstetric history		
Description of each previous pregnancy	95	±2
Gestational age of each previous delivery	82	±3
Birth weight of each previous delivery	93	±2
Complications of each previous pregnancy	80	±3
Entry for each previous abortion	93	±2
Gestational age of each previous abortion	82	±3
Behavioral history		
Occupation	63	±3
Work outside home	41	±3
Social problems	27	±3
Major life events	04	±1
Intrafamily problems	23	±3
Financial stress	06	±2
Alcohol use	51	±3
Alcohol abuse	24	±3
Drug abuse or addiction	44	±3
Smoking	59	±3
Quantity of cigarettes smoked	25	±3
Change in consumption of cigarettes during pregnancy	02	±1
Initial, serial pregnancy surveillance		
Last menstrual period	98	±1
Date of quickening	59	±3
Uterine size at first visit	53	±3
Nutritional assessment	38	±3
Flow chart with entries for each visit		
Weight	98	±1
Fundal height	95	±2
Gestational age	69	±3
Date	98	±1
Patient education and special referrals		
General patient education	38	±3
Childbirth education	35	±3
Nutrition education	19	±3
Referrals for education or other health care (e.g., level III facility)	13	±2
Complications, developing problems		
Indicated space to record complications	51	±3
Special checklist with entry for intrauterine growth retardation	24	±3
Special checklist with entry for ≥ 1 episodes of preterm labor	07	±2
Special checklist with entry for sexually transmitted disease	25	±3
List of risks in present pregnancy		
Checklist for risk assessment	30	±3
Laboratory tests		
Syphilis test results	96	±1
Herpes culture results	16	±2
Chlamydia test results	03	±1
One or more spaces for bacteruria results	63	±3
Two or more spaces for hemoglobin or hematocrit results	77	±3
Other diagnostic procedures		
Ultrasonography performed	39	±3
Date of ultrasonography	26	±3
Indication for ultrasonography	10	±2
Therapeutic procedures		
Prenatal hospitalization	04	±1
Prescribed bedrest	01	±0.4
Problem-specific education or advice	19	±2
Other treatments	50	±3
Medications	66	±3

*95% Half-widths were computed as 1.96 times the SE of the estimated percentage of records with the item or indicator present.

Table V. Weighted estimates of the percentage of prenatal records with selected items (dependent variables) by characteristics of respondents (independent variables)

Independent variable	*Dependent variable*										*Sample Size*	*95% Half-width†*		
	DEMO	*DRINK*	*SMOKE*	*USIZE*	*NUTR*	*EDUC*	*IUGR*	*STD*	*RISK*	*MEDIC*		*Minimum*	*Maximum*	*Mean*
Type of record														
POPRAS, Hollister	100	89*	89*	100	100	100	100	100	100	100	153	0.0	4.8	1.0
Other	23	44	13	44	26	25	09	10	17	60	814	2.1	3.5	2.9
Primary specialty														
OB/GYN	35	53	25	56	39	39	23	22	29	70	840	2.9	3.4	3.2
Other	38	46	27	43	36	33	29	35	35	52	129	8.2	9.1	8.7
Type of practice														
Private solo, group, or HMO	33	50	23	52	35	36	22	23	29	65	900	2.9	3.4	3.2
Other	63	68	53	68	73	55	49	54	54	80	59	10.4	13.0	12.3
Time since graduation from medical school (yr)														
≤ 15	36	58	30	58	39	42	28	28	33	72	388	4.7	5.1	4.9
> 15	35	47	23	50	38	35	22	23	29	63	579	3.6	4.2	3.9
Geographic region														
Northeast, California	35	53	23	51	36	35	23	22	29	66	374	4.7	5.4	5.1
Other	36	50	27	55	40	39	25	27	32	67	595	3.5	4.1	3.8

See Appendix for explanation of table headings.

*1985 and 1987 POPRAS records included this item; earlier versions did not.

†95% Half-widths were computed as 1.96 times the SE of the estimated percentage of records with the item or indicator present.

medical school. Physicians who were engaged in private or HMO practices used substantially fewer records with each of the items of interest. Similarly, smaller percentages of records of physicians who finished medical school more than 15 years before the survey included the items when compared with those of physicians who had been practicing for 15 or fewer years.

Each of the items in Table V was analyzed in a series of logistic regression models with physician specialty group, the type of practice in which the physician was engaged, the number of years in practice, and the geographic location of the practice as independent variables. The type of prenatal record was not generally useful for this analysis because, except for quantity of cigarettes smoked and use of alcohol, all the physicians who used Hollister and POPRAS standardized forms had the items present. Because of the colinearity created by the type of prenatal record, the coefficients with the type of prenatal record present are little different from those with the type of prenatal record absent. This analysis was thus done on the subset of respondents (84.2%) who did not use the POPRAS or Hollister forms.

Our analysis strategy was to subject all 10 items to the same models. We first examined predictive equations with both main and interaction effects present. This approach afforded us the most direct comparison of the predictive nature of the independent variables among dependent variables as it clarified the relatively minor role of higher-order interaction effects. For the 10 models that involved the 4 independent variables previously listed, only 3 of 60 first-order interaction coefficients were statistically significant (at the $p < 0.05$ level).

Findings for the main effects models presented in Table VI generally confirm our earlier descriptive findings. The type of practice was clearly important statistically because coefficients for the type of practice were found to be significant in 7 of 10 models. The negative sign in each case indicates that, with other independent effects constant, those physicians in HMOs and in private practice were relatively less likely to have labeled spaces for recording these items than were physicians in other types of practices.

All but two coefficients for the number of years since medical school were positive, and three of them (use of alcohol, checklist with intrauterine growth retarda-

Table VI. Logistic regression coefficients for main effects models for the presence of selected items in physicians' prenatal records (excluding FORM as an independent variable)

	Dependent variable									
	DEMO	*DRINK*	*SMOKE*	*USIZE*	*NUTR*	*EDUC*	*IUGR*	*STD*	*RISK*	*MEDIC*
Sample size	806	808	806	807	804	805	806	806	799	804
Model correlation	0.07	0.12	0.18	0.05	0.12	0.05	0.19	0.22	0.06	0.10
Intercept	−0.05	−0.00	−0.79	−0.48	0.35	−1.26	−1.10	−0.09	−0.61	0.19
Main effect										
Specialty	−0.25	0.29	0.42	0.55*	0.14	0.60	−0.69	−1.12‡	−0.32	0.59*
Practice	−1.06‡	−0.79†	−1.74‡	−0.30	−1.51‡	−0.48	−1.30‡	−1.58‡	−0.96‡	−0.47
Years	−0.12	0.51‡	0.39*	0.25*	−0.21	0.28*	0.60†	0.29	0.14	0.42‡
Location	0.16	0.26*	−0.10	0.01	−0.02	0.11	0.61†	0.23	0.22	0.15

See Appendix for explanation of table headings.
*$0.10 > p > 0.05$.
†$0.05 > p > 0.01$.
‡$0.01 > p$.

tion, and use of medications) were statistically significant (at the $p < 0.05$ level). This suggests that physicians who were recent graduates (≤15 years) were more likely to use records with entries for behavioral and other risks. For each of the remaining independent variables (specialty and location) only one coefficient was significant. Physicians who did not list obstetrics and gynecology as a primary specialty used significantly more records with checklists that included an entry for sexually transmitted diseases. Those in the Northeast and in California used more records with entries for alcohol use. Otherwise, specialty and location were not strongly related to the dependent variables.

Comment

Despite compelling reasons for using detailed systematic records in prenatal care, the results of this national survey show that such records are not in widespread use. Most of the records in this sample included items of traditional medical and obstetric significance, the recording of which contributes to the essential functions of prenatal records (Table I). In general, however, the level of detail in the records did not adequately reflect the emergence of new information about risks of adverse pregnancy outcomes or newer technologies for prenatal surveillance and diagnosis of problems.

The commercially available POPRAS and Hollister records included virtually all the items of interest, although some respondents were using older POPRAS forms that did not include the smoking and drinking items required for this analysis. The availability of entries in the records developed by others varied greatly. It is important to note, however, that some of the records in the "other" group were detailed and systematic, as shown by the results of logistic regression analysis, which excluded respondents who used POPRAS and Hollister forms.

Logistic regression analysis also showed that the physicians who were more likely to have detailed record items were in hospital and government program-based practices and were recent (≤15 years) graduates of medical schools. Medical schools are, of course, the locations of many hospital-based practices.

The types of prenatal record entries that were found deficient in our study were also identified in the Newborn Neurologic Deficit Study (Localio AR and Siegel JE. Unpublished data.). In their analysis of 591 claims involving allegations of neurologic deficit caused by negligent medical care for deliveries, the investigators found that only 15% reported information on alcohol consumption and that 27% reported smoking history. They also observed that whereas prenatal diagnostic tests and procedures were better documented, the results were frequently unavailable. In an effort to apply a simplified risk scoring system, they found that data on at least one risk factor were missing in 45% of the cases. From a legal perspective, these deficiencies, which are highly consistent with the ones we found, leave practitioners extremely vulnerable in the event of malpractice litigation.

Item deficiencies also undermine the ability of prenatal records to serve other functions adequately. In a less complicated time, when a single physician provided continuous care to his or her patients, simple records with adequate space for comments were probably sufficient. Many of the records we received had ample space for "write-ins." Without clear labels, however, the information necessary for adequate patient care in the 1990s, which includes billing, quality assurance, and communication within and among health care systems, may or may not be recorded.

On the other hand, it cannot be assumed that the presence of labeled spaces in a medical record will assure that the requested entries are made. Incomplete and misleading entries in medical records have been documented repeatedly,[15, 16] and the correlation between good reporting and good care remains controversial.[15] However, without specific labels and adequate detail, the extent to which recording may be incomplete is difficult to assess. The purpose of the systematic de-

sign of problem-oriented medical records is to promote completeness and to reduce ambiguity in medical recordings.[17] The POPRAS and Hollister forms, as well as many of the records developed by hospitals and government programs, are based on the problem-oriented medical record model. As indicated, the American College of Obstetricians and Gynecologists has recently begun to distribute a new systematic record.[10] Most of the record is organized as a checklist with space for comments. Special emphasis has been given to linking diagnostic tests to procedures with corresponding results and interpretations. Whereas the form does not include all of the items examined in this study, it does include most of them, and its four-page format may be attractive to busy practitioners.

Physicians in private practice (solo or group), those associated with HMOs, and those who have been practicing for more than 15 years may wish to review the adequacy of their records with regard to the functions that prenatal records should serve, the results of this study, and the options for detailed systematic records that are available to them.

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Appendix

Definitions of items from the National Prenatal Records Survey

Item	*Description (categories used for logistic regression analysis)*
Dependent variables (1 = item present in record, 0 = item not present)	
DEMO	Basic minimum sociodemographic data (i.e., age, race, education, and marital status)
DRINK	Space to indicate alcohol use in present pregnancy
SMOKE	Indication of the quantity of cigarettes smoked
USIZE	Space to record uterine size at first prenatal visit
NUTR	Space to indicate that a nutritional assessment was done
EDUC	Space to document general patient education
IUGR	Checklist of complications of present pregnancy with an entry for intrauterine growth retardation
STD	Checklist of complications of present pregnancy with an entry for sexually transmitted diseases
RISK	Checklist for the purpose of risk assessment
MEDIC	Space for recording medications used in current pregnancy
Independent variables	
FORM	Type of prenatal record (1 = POPRAS/Hollister standardized form, 0 = other)
SPECIALTY	Physician's primary specialty (1 = obstetrics and gynecology, 0 = other)
PRACTICE	Type of physician practice (1 = private solo or group, HMO, 0 = other)
YEARS	Number of years since graduation from medical school (1 = ≤15 years, 0 = >15 years)
LOCATION	Region of the country as determined by the first digit of the zip code for the address of the physician's practice (1 = first digit of 0, 1, or 9 for the Northeast and California; 0 = other)

Reproductive factors and risk of endometrial cancer

Fabio Parazzini, MD,[a] Carlo La Vecchia, MD,[a, b] Eva Negri, ScD,[a] Luigi Fedele, MD,[c] and Fulvia Balotta, MD[a]

Milan, Italy, and Lausanne, Switzerland

The role of reproductive factors in endometrial cancer risk has been analyzed in a case-control study conducted since 1983 in the greater Milan area on 568 women (cases) with histologically confirmed endometrial cancer and 1925 women (controls) who were admitted for acute, nonmalignant, hormonal, gynecologic conditions to hospitals that cover a comparable catchment area. Compared with nulliparous women, parous women had a 30% lower risk of endometrial cancer, but there was no evidence of a decline in risk with increasing number of births. The risk of the disease decreased with number of spontaneous or induced abortions; the multivariate relative risk estimates were, compared respectively with no spontaneous or induced abortions, 0.5 for women with two or more spontaneous abortions and 0.3 for women with two or more induced abortions; both trends in risk were statistically significant. When parous women only were considered, no association emerged between endometrial cancer and age at first birth, but the risk decreased with increasing age at last birth: compared with women whose last birth occurred before age 25, the relative risk was 0.5 for women who were ≥35 years old at last birth, and the multivariate trend in risk was statistically significant. For most of the reproductive factors that were considered, the risk estimates tended to be greater at younger age or among premenopausal women and to flatten off in subsequent strata of age. An association between endometrial cancer and age at first birth was observed in women who were ≤49 years old, but not in older groups. The observation that later age at last birth as well as later first birth in younger women decreases the risk of endometrial cancer suggests a short-term protective effect of pregnancy. This finding is consistent with a late-stage (promotional) effect of reproductive factors on endometrial carcinogenesis. (Am J Obstet Gynecol 1991;164:522-7.)

Key words: Endometrial cancer, reproductive factors

Reports from different countries have suggested that infertile or nulliparous women are at increased risk of endometrial cancer. The potential protective role of each single birth or total number of births is, however, largely controversial.[1-12] For example, a decreasing trend in risk of endometrial cancer with increasing number of term pregnancies was observed in two case-control studies conducted in the United States[4, 5] and in a cohort study in Norway[10] but not in a previous analysis of our Italian data set[6] and in an American case-control study.[1] Furthermore, the extent of protection, if any, of incomplete pregnancies (i.e., spontaneous or induced abortions) is unclear.[2, 5, 10] Recently a Norwegian cohort study[10] and a Scandinavian population-based case-control study[9] have shown that endometrial cancer was more frequent in women of early age at first and last birth, which suggests that the relation between parity and risk of endometrial cancer shares a more complex hormonal mechanism than infertility. We have further considered the relation between reproductive factors and the risk of endometrial cancer with data from a large case-control study that was conducted in Italy.

Patients and methods

In the framework of a general surveillance of breast and gynecologic malignancies, since 1983 we have been conducting a hospital-based case-control study of endometrial cancer in the greater Milan area.[6, 13, 14]

The cases that were studied were 568 women below the age of 75 (range, 28 to 74 years; median, 61 years) with histologically confirmed endometrial cancer that was diagnosed within the year that preceded the interview who had been admitted to the National Cancer Institute and to the Ospedale Maggiore (which includes the four largest teaching and general hospitals in the greater Milan area).

Controls were women who were admitted because of acute conditions to several specialized university clinics or to the Ospedale Maggiore of Milan. They were admitted because of diseases that were not malignant,

From the Istituto di Ricerche Farmacologiche "Mario Negri," Milano,[a] Institute of Social and Preventive Medicine, University of Lausanne,[b] and the Prima Clinica Ostetrica-Ginecologica, Università di Milano.[c]

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Reprint requests: Fabio Parazzini, MD, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea, 62, 20157 Milano, Italy.

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Table I. Distribution of 568 cases of endometrial cancer and 1925 controls according to selected characteristics (Milan, Italy, 1983 to 1988)

Patient characteristics	*Endometrial cancer*		*Controls*	
	No.	*%*	*No.*	*%*
Age (yr)				
<40	11	1.9	325	16.9
40-49	55	9.7	444	23.1
50-59	178	31.3	521	27.1
60-69	192	33.8	442	23.0
≥70	132	23.2	193	10.0
Education (yr)				
<7	427	75.2	1159	60.2
7-11	99	17.4	453	23.5
>11	42	7.4	313	16.3
Marital status				
Ever married	493	86.8	1662	86.3
Never married	75	13.2	263	13.7
Menopausal status				
Premenopausal	103	18.1	817	42.4
Postmenopausal	465	81.9	1108	57.6
Body mass index (kg^{-2})				
<25	213	37.5	1178	61.2
25-29	197	34.7	565	29.4
>29	158	27.8	182	9.5
Estrogen replacement therapy				
Yes	81	14.3	61	3.2
No	487	85.7	1864	96.8

hormonal, or gynecologic in origin and/or that were judged to be unrelated to any of the identified or suggested risk factors for endometrial cancer. They had not undergone hysterectomy and/or bilateral oophorectomy. A total of 1925 controls, aged 25 to 74 (median age, 54), were interviewed; of those, 32% were admitted because of traumatic conditions (mostly fractures and sprains), 25% had nontraumatic orthopedic disorders (mostly low back pain and disc disorders), 15% had conditions that required surgery (mostly abdominal, such as acute appendicitis or strangulated hernia), and 28% had other illnesses, such as ear, nose and throat or teeth disorders. Less than 2% of the identified cases and controls refused to be interviewed.

Information was collected by trained interviewers with a standard questionnaire on personal characteristics and habits, gynecologic data, number of births, number of spontaneous and induced abortions, age at first and last births, relevant medical history, and history of lifetime use of female hormones. The present report is based on data that were collected up to December 1988.

Data analysis. Odds ratios (as estimators of relative risks) of endometrial cancer together with their 95% approximate confidence intervals were first computed from data stratified in 5-year intervals of age by the Mantel-Haenszel procedure.[15] When a factor could be classified in more than two levels, the significance of the linear trend was assessed by the test described by Mantel.[16] Relative risks were then determined after simultaneous allowance for potential confounding effects of age (in single years), education, marital status, menopausal status, body mass index, estrogen replacement therapy, hypertension, diabetes, and, in turn, parity, number of induced and spontaneous abortions, and age at first and last birth through unconditional multiple logistic regression equations that were fitted by the method of maximum likelihood.[17]

Results

The distribution of cases and controls according to age, education, marital status, menopausal status, body mass index, and use of estrogen replacement therapy is presented in Table I. The control group was consistently younger than the case group (median age, 54 vs 61 years), which reflects the criteria of selection within this case-control surveillance that covers simultaneously several gynecologic neoplasms. Furthermore, women with endometrial cancer tended to be less educated and more frequently overweight, and they reported more frequent use of estrogen replacement therapy.

Overall, compared with nulliparous women, parous women had a 30% lower risk of endometrial cancer. The multivariate point estimate was of borderline statistical significance. There was no evidence, however, of a decline in risk with increasing number of births, and the test for trend was not statistically significant (Table II). The risk of cancer of the corpus uteri decreased with number of spontaneous or induced abor-

Table II. Relative risk of endometrial cancer according to selected reproductive factors (Milan, Italy, 1983 to 1988)

Variable	*Endometrial cancer*	*Controls*	*M-H**		*MLR†*	
			RR	*95% CI*	*RR*	*95% CI*
Parity						
0	128	394	1‡§		1‡	
1	120	460	0.8	0.6-1.1	0.7	0.5-1.0
2	158	589	0.9	0.6-1.1	0.7	0.5-1.1
≥3	161	482	0.8	0.6-1.1	0.7	0.5-1.0
Total parity ≥ 1	431	1351	0.8	0.7-1.1	0.7	0.5-1.0
χ^2_1 trend				0.1		1.3
Spontaneous abortions						
0	458	1510	1‡		1‡	
1	81	264	1.0	0.7-1.3	0.9	0.7-1.3
≥2	29	151	0.6	0.4-0.9	0.5	0.3-0.8
χ^2_1 trend				5.1‖		7.8¶
Induced abortions						
0	543	1695	1‡		1‡	
1	13	121	0.5	0.3-0.9	0.5	0.3-1.0
≥2	12	109	0.3	0.2-0.5	0.3	0.2-0.7
χ^2_1 trend				12.0¶		15.6¶
Age at first birth (yr)						
≤24	202	739	1‡		1‡	
25-29	175	539	1.1	0.8-1.4	1.1	0.8-1.4
≥30	62	250	0.7	0.5-1.0	0.7	0.5-1.0
χ^2_1 trend				1.3		1.6
Age at last birth (yr)						
≤24	67	226	1‡		1‡	
25-29	121	494	0.7	0.5-1.0	0.7	0.5-1.0
30-34	137	448	0.8	0.5-1.1	0.6	0.4-0.9
≥35	113	359	0.7	0.4-1.1	0.5	0.3-0.8
χ^2_1 trend				3.8‖		7.5¶

RR, Relative risk; *95% CI*, 95% confidence interval.

*Mantel-Haenszel estimates adjusted for age.

†Derived from logistic model including terms for age, education, marital status, menopausal status, body mass index, estrogen replacement therapy, hypertension, diabetes, and, in turn, the above-listed variables.

‡Reference category.

§Adjusted for age and marital status.

‖$p < 0.05$.

¶$p < 0.01$.

tions; the relative risk estimates compared respectively with no spontaneous or induced abortions, were 0.6 for women with two or more spontaneous abortions and 0.3 for women with two or more induced abortions. Both trends in risk were statistically significant.

When parous women only were considered, no significant association emerged between endometrial cancer and age at first birth. Compared with women whose first birth occurred before the age of 25, the relative risks were 1.1 for women 25 to 29 years old and 0.7 for women ≥30 years old. In contrast, the risk of the disease decreased with increasing age at last birth: compared with women whose last birth occurred before the age of 25, the multivariate relative risks were 0.7 for women 25 to 29 years old, 0.6 for women 30 to 34 years old, and 0.5 for women ≥35 years old. The multivariate relative risks for various reproductive variables were largely similar to the Mantel-Haenszel estimates, which were adjusted for age only (Table II).

The role of reproductive factors was further examined in strata of menopausal status (Table III). Compared with nulliparous women, the risk in parous women was 0.3 (95% confidence interval = 0.1 to 0.6) before menopause and 0.8 (95% confidence interval = 0.6 to 1.2) after menopause. Spontaneous and induced abortions and age at last birth were inversely related with the risk of cancer of the corpus uteri in premenopausal and postmenopausal women, but these inverse associations tended to be systematically stronger in premenopausal women. The relative risk for two or more miscarriages, for instance, was 0.3 before menopause and 0.5 after menopause, and relative risk for

Table III. Multivariate relative risk* of endometrial cancer according to selected reproductive factors and menopausal status (Milan, Italy, 1983 to 1988)

	Menopausal status			
	Before menopause		*After menopause*	
Variable	*RR*	*95% CI*	*RR*	*95% CI*
Parity				
0	1†		1†	
1	0.2	0.1-0.6	0.9	0.6-1.3
2	0.3	0.1-0.6	0.9	0.6-1.4
≥3	0.4	0.2-1.0	0.8	0.5-1.2
Total parity ≥1	0.3	0.1-0.6	0.8	0.6-1.2
χ^2_1 trend	0.4		1.4	
Spontaneous abortions				
0	1†		1†	
1	0.8	0.4-1.6	1.0	0.7-1.4
≥2	0.3	0.1-1.0	0.5	0.3-0.9
χ^2_1 trend	3.9‡		4.5‡	
Induced abortions				
0	1†		1†	
1	0.5	0.2-1.6	0.5	0.2-1.0
≥2		NE	0.5	0.2-1.0
χ^2_1 trend	11.9§		7.2§	
Age at first birth (yr)				
≤24	1†		1†	
25-29	0.7	0.4-1.2	1.2	0.9-1.7
≥30	0.7	0.3-1.6	0.7	0.0-1.1
χ^2_1 trend	1.6		0.7	
Age at last birth (yr)				
≤24	1†		1†	
25-29	0.3	0.1-0.7	0.9	0.6-1.4
30-34	0.3	0.1-0.7	0.7	0.5-1.2
≥35	0.3	0.1-0.7	0.6	0.4-1.1
χ^2_1 trend	4.9‡		3.7‡	

RR, Relative risk; *95% CI*, 95% confidence interval; *NE*, not estimated.

*Derived from logistic model including terms for age, education, marital status, body mass index, estrogen replacement therapy, hypertension, diabetes and, in turn, the above-listed variables.

†Reference category.

‡$p < 0.05$.

§$p < 0.01$.

last birth at age ≥35 was 0.3 before menopause and 0.6 after menopause.

Corresponding analyses in subsequent strata of age (<50, 50 to 59, and ≥60) are presented in Table IV. For various reproductive factors that were considered, the associations tended to be stronger at younger ages and to flatten off in following strata of age, even after allowance for menopausal status by means of multivariate analysis. For instance, the relative risks for parous versus nulliparous women were 0.3, 0.6, and 0.8 in the three age groups that were considered.

An association between endometrial cancer and age at first birth was observed in women ≤49 years old (compared with women who had first birth at ≤24 years old, the multivariate relative risk estimates were 0.6 and 0.4 for women 25 to 29 years old and ≥30 years old) but not in older subgroups. Likewise, in younger women the risk of endometrial cancer decreased with late age at last birth, but this association tended to flatten off in subsequent strata of age.

Comment

This large data set provides more precise information than has been previously available on the role of reproductive factors in endometrial carcinogenesis. A reduction in risk (of borderline significance) was observed in parous women, but there was no evidence for the risk to decline with increasing numbers of births. Spontaneous or induced abortions and late age at last birth appear to protect against endometrial cancer. These effects were more evident in premenopausal women. Late age at first birth was associated with a

Table IV. Multivariate relative risk* of endometrial cancer according to selected reproductive factors in subsequent age group (Milan, Italy, 1983 to 1988)

	Age group (yr)					
	≤49		*50-59*		*≥60*	
Variables	*RR*	*95% CI*	*RR*	*95% CI*	*RR*	*95% CI*
Parity						
0	1†		1†		1†	
1	0.3	0.1-0.8	0.6	0.3-1.3	0.8	0.5-1.4
2	0.3	0.1-0.6	0.6	0.3-1.3	1.0	0.6-1.6
≥3	0.4	0.1-1.0	0.6	0.3-1.3	0.8	0.5-1.3
Total parity ≥1	0.3	0.1-0.7	0.6	0.3-1.2	0.8	0.5-1.3
χ^2_1 trend	1.7		0.5		0.4	
Spontaneous abortions						
0	1†		1†		1†	
1	0.6	0.2-1.4	1.1	0.7-1.9	1.0	0.6-1.5
≥2	0.3	0.0-1.3	0.6	0.3-1.3	0.5	0.2-0.9
χ^2_1 trend	4.8‡		0.8		4.2‡	
Induced abortions						
0	1†		1†		1†	
1	0.6	0.2-2.0	0.4	0.1-1.2	0.6	0.2-1.8
≥2	0.1	0.0-0.9	0.7	0.3-1.9	0.2	0.0-0.7
χ^2_1 trend	7.4§		1.7		8.5§	
Age at first birth (yr)						
≤24	1†		1†		1†	
25-29	0.6	0.3-1.2	1.1	0.7-1.8	1.3	0.9-1.9
≥30	0.4	0.1-1.4	0.7	0.4-1.3	0.8	0.5-1.3
χ^2_1 trend	3.2		0.7		0.0	
Age at last birth (yr)						
≤24	1†		1†		1†	
25-29	0.3	0.1-0.8	0.6	0.3-1.2	1.1	0.6-2.0
30-34	0.3	0.1-0.8	0.5	0.2-1.0	0.9	0.5-1.7
≥35	0.3	0.0-1.0	0.3	0.1-0.7	1.0	0.5-1.9
χ^2_1 trend	4.1†		8.9§		0.1	

RR, Relative risk; *95% CI*, 95% confidence interval.

*Derived from logistic model including terms for age, education, marital status, menopausal status, body mass index, estrogen replacement therapy, hypertension, diabetes and, in turn, the above-listed variables.

†Reference category.

‡$p < 0.05$.

§$p < 0.01$.

lower risk of cancer of the corpus uteri in women ≤49 years old, but the relation tended to flatten off in older women. Thus age or menopausal status appeared to have an important modifying effect on the relation between reproductive factors and risk of endometrial cancer.

Selection should not present a major problem in this study, since cases and controls were identified in institutions that cover broadly comparable catchment areas and since participation was almost complete. Likewise, information bias can hardly have a role on variables like parity or age at births. In relation to confounding, strict allowance for single year, as well as simultaneous allowance for several potential distorting factors, including body mass index, measures of social class, or marital status, did not appreciably change the risks for any of the reproductive factors that were considered. The role of reproductive variables should be considered in the framework of the unopposed estrogen hypothesis in endometrial carcinogenesis,[18] which postulates that any factors that increase esposure of endometrium to unopposed estrogen increase the risk of the disease. Conversely, anything that increases progesterone levels would lower the risk.[18-20]

Pregnancy reduces the time of exposure to unopposed estrogens and this may explain the higher risk in nulliparous women. However, in our study the protective effect of parity seems to be largely restricted to the first term pregnancy; the risk estimates are comparable in subsequent strata of number of births. This suggests that infertility and not parity per se should be associated with the risk of endometrial cancer. Infertility can be related to anovulation, which is characterized by long-term unopposed estrogenic menstrual cycles.[18, 21]

The hypothesis that infertility is a risk factor for endometrial cancer is supported by studies that show a higher risk for married nulliparous women than for unmarried ones.[2] Furthermore, two case-control studies and a cohort study that were conducted in the United States found an increased risk of endometrial cancer in women who reported infertility prob-

lems.[5, 7, 22] In a cohort study of infertile women, however, there was no overall increased incidence of endometrial cancer, although the risk was apparently elevated in women <50 years old or in the first 20 years after evaluation.[23] In the latter study, the risk was still greater in women with progesterone deficiency.

Few studies have analyzed the relation between incomplete pregnancies and risk of endometrial cancer, and the findings are largely inconsistent.[2, 5, 10] In our study, the risk of the disease was significantly lower in women who reported one or more spontaneous or induced abortions. This can be interpreted in terms of differential fertility between endometrial cancer cases and controls. The decreasing trend in risk that was observed with increasing numbers of abortions could be tentatively viewed in terms of higher fertility in women who required induced abortions or who reported more frequent miscarriages, among which a certain proportion of induced abortions may possibly be misclassified.

The finding that the effect of nulliparity is more evident in premenopausal women and the significant inverse association with age at last birth that was found in this case-control study, as well as in a prospective study that was conducted in northern Europe,[10] suggest that the protective effect of pregnancy is greater in the short term after birth. This is consistent with the pattern of risk in infertile women in the Mayo Clinic cohort.[23] Along this line, we have found that later age at first birth decreased the risk in younger women (i.e., up to one or two decades after pregnancy) and that this effect tended to flatten off in subsequent age groups. Within the framework of the multistage theory of carcinogenesis,[24] this is consistent with a late-stage (promotional) effect of these reproductive factors, which is similar to that observed for estrogen replacement treatment.[18] This again confirms that the biologic mechanisms for the action of these reproductive factors is probably mediated through changed hormonal balances and thus contributes to a unifying interpretation of the role of various risk factors for endometrial carcinogenesis.

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Cervical findings in rape victims

Laura Slaughter, MD and Carl R.V. Brown, PhD
San Luis Obispo, California

The difficulty of documenting genital findings in rape victims by gross visualization is well known. However, we report two cases in which cervical findings were readily established by colposcopic examination and photography. Use of the colposcope may significantly increase the reliability of establishing findings in rape victims. (Am J Obstet Gynecol 1991;164:528-9.)

Key words: Physical findings, rape; cervical trauma, sexual assault

Obtaining corroborative physical data from rape victims is a difficult task. Previously published literature documents only a 10% to 30% rate of positive physical findings in such cases.[1, 2] This dismal result has prompted some authors to suggest that the performance of a genital examination be reserved for those who complain of pain or have a history of undergoing instrumentation.[2] However, with the magnification made possible by using the colposcope, we report two cases in which cervical findings were documented and discuss their medical and forensic significance.

Case reports

Case 1. A 17-year-old female was invited by a friend to his room in a fraternity house. There he immobilized her on his bed and put his fingers into her vagina and began "jabbing her." She began crying; he eventually stopped and allowed her to leave. A physical examination was performed 5 days after the incident. With the colposcope, punctate areas of hemorrhage were seen on the hymen at 5 and 7 o'clock with the patient in a supine position. Cervical examination showed fine linear abrasions just below the cervical os (Fig. 1). Menstrual blood was noted. Reevaluation in 5 weeks showed complete healing of these injuries.

Case 2. A 30-year-old woman presented to the emergency room with complaints of lower abdominal pain and scanty vaginal bleeding. Approximately 2 hours earlier, she had been attacked by an unknown man while walking on the beach. After pinning her down, he stuck what "felt like a knife" into her vagina. Genital examination showed blood at the introitus. A linear laceration was noted on the right in the vaginal barrel, and the cervix showed several punctate and linear lacerations (Fig. 2). These were completely healed by the time of her follow-up examination in 4 days.

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Fig. 1. Fine linear abrasion on inferior surface of cervix.

Comment

The medical literature to date establishes that genital examination in rape victims yields results in 10% to 30% of cases. This information is based on examination by means of gross visualization alone. In contrast, applying the advanced technology afforded by the colposcope, our group was able to see and photograph trauma to the victim's cervix. This type of injury has not been previously reported. These two cases illustrate how trauma can be reliably documented via colposcopic magnification; in both, the pattern of injury is inconsistent with blunt force or penile penetration. Moreover, documentation of the healing process is an important part of the forensic assessment. First, it links the traumatic event to the injuries observed, establish-



Fig. 2. Multiple puncture wounds and fine linear lacerations on superior surface of cervix.

ing a time frame reliably associated with injury. Second, documentation of the victim's normal anatomy in the follow-up examination effectively establishes her as her own control against which the court can evaluate the nature, extent, and cause of the injury. This is particularly important when the body parts injured are not ones commonly seen. Neither of these patients needed treatment, and thus the injuries may be considered trivial from a purely medical viewpoint. Legally, the information obtained from these examinations is significant. The data here support vaginal penetration, which is basic to a charge of rape. In addition, it can also substantiate the use of a foreign object,* which is a separate crime and thus offers an enhancement in charging and sentencing procedures.

The physician who examines rape victims must provide not only medical and psychological support to the victim but also forensic information to the legal authorities. This information must include evidence of vaginal penetration and physical findings consistent with the history and timing given by the victim. Protocols to examine victims of sexual assault might be revised to include colposcopy to more reliably document genital injuries and their resolution.

We gratefully acknowledge the efforts of Jane Kulick, RN, and the San Luis Obispo County Suspected Abuse Response Team and express our appreciation for the support of San Luis Obispo General Hospital and Sexual Assault Victim Education.

*Rape with a foreign object is defined by the California Penal Code as vaginal penetration with anything other than a penis.

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Continuous electrocardiographic monitoring in hypertensive crises in pregnancy

Datshana P. Naidoo, MD, Ismail Bhorat, MD, Jack Moodley, MD, Janardana K. Naidoo, MD, and Abdul S. Mitha, MD

Durban, South Africa

Twenty-four patients first seen with hypertensive crises during pregnancy were studied by continuous electrocardiographic monitoring for a period of 24 hours to detect the presence of serious ventricular arrhythmias. Three patients were excluded from analysis because of low serum potassium levels. Thirteen of the remaining 21 patients had ventricular tachycardia on subsequent analysis of the electrocardiogram. These arrhythmias subsided after induction of anesthesia when blood pressure control was optimal. This finding may be implicated in the pathogenesis of pulmonary edema and sudden death in these patients. (AM J OBSTET GYNECOL 1991;164:530-3.)

Key words: Arrhythmia, hypertension, pregnancy

Eclampsia is the leading cause of maternal mortality at large referral centers throughout the world.[1] In 1987 seven of 34 maternal deaths at the King Edward VIII Hospital in Durban, South Africa, were caused by eclampsia. Although cerebral hemorrhage does cause a significant number of these deaths, a proportion of them are unexplained. Furthermore, pulmonary edema is a recognized complication in patients with hypertensive crises of pregnancy and contributes to increased mortality in these patients.[2] The possibility that unexplained sudden death and pulmonary edema may be related to serious cardiac arrythmias has not been investigated. The aim of this study was to detect the presence of malignant ventricular arrhythmia in patients with hypertensive crises of pregnancy.

Material and methods

Selection criteria. The study group comprised 24 patients in whom the diagnoses of eclampsia and imminent eclampsia were made. Eclampsia was defined as convulsions associated with hypertension and proteinuria. A diagnosis of imminent eclampsia was made in patients with hypertension, proteinuria, and symptoms of imminent eclampsia (i.e., epigastric pain, blurring of vision, headache, and cerebral irritability).[3] All patients were managed by standard procedures described elsewhere.[4] This included central venous pressure monitoring, lowering of blood pressure with intravenous hydralazine hydrochloride, diazepam to terminate seizure, and magnesium sulfate to prevent further convulsions. The time of administration of specific drug therapy including those used during anesthesia were recorded. In patients who required cesarean section a standard regimen of drug administration was used. This included intravenous administration of lidocaine (100 mg) to suppress a hypertensive response during intubation.[4]

On entry into the study, a full clinical examination was performed and blood samples were taken for measurement of serum potassium, magnesium, and calcium. An arterial blood sample was obtained for blood gas analysis and radiography of the chest was performed. Continuous electrocardiographic monitoring was then instituted for a period of 24 hours to include the predelivery, delivery, and immediate postdelivery periods. Recording was performed with a four-channel Holter recorder (Spacelab 7200, Redmond, Washington) and the full recording was printed for analysis. Arrhythmia detection and analysis were performed on a Holter analysis computer (Spacelab 90101). Results were confirmed by visual inspection of the complete recording and ventricular extrasystoles (premature ventricular contraction) were analyzed by the modified Lown grading system.[5] The grading system assigns premature beats into one of the following categories: grade 0, not a single premature ventricular contraction in 24 hours; grade I, occasional premature ventricular contractions; grade II, frequent premature ventricular contractions (>30 per hour or 1 per minute); grade III, multiform premature ventricular contractions;

From the Medical School, University of Natal.
Received for publication June 16, 1990; accepted September 14, 1990.
Reprint requests: Dr. D. P. Naidoo, Medical School, University of Natal, P. O. Box 17039, Congella, South Africa 4013.
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grade IVA, couplets; grade IVB, runs of three or more in succession (ventricular tachycardia).

Control readings were obtained in normotensive primigravid women in the last trimester of pregnancy. Three patients with low serum potassium levels were excluded from analysis. In the remaining patients serum biochemistry and blood gas values were within normal limits. The mean age in both study and control groups was 22 years. Inasmuch as most of the patients with imminent eclampsia–eclampsia syndrome arrive at the hospital early, the mean period of gestation in the study group was 32 weeks (control = 38 weeks). Sixteen patients in the study group and eight in the control group were delivered of infants by cesarean section. The remainder were delivered vaginally.

For statistical purposes each grade was assigned a numeric score that ranged from 1 (grade 0) to 6 (grade IVB). Both the frequency of premature ventricular contractions and grade of arrhythmia was compared in the predelivery and delivery phases and in the delivery and postdelivery phases (Wilcoxon signed-rank test). The grade of arrhythmia was compared with mean arterial pressure with the nonparametric Mann-Whitney U test.

Results

In the study group the mean of the systolic, diastolic, and mean arterial pressures on admission were 174, 120, and 138 mm Hg, respectively (control group: 110, 70, and 83 mm Hg, respectively). Precordial examination was normal in both groups. A third heart sound was heard in three patients in the study group. Cardiomegaly was not a feature in any of these patients. Three patients who had crepitations at the lung bases exhibited pulmonary edema on chest radiography. Central venous pressure was raised to 16 cm water in these three patients.

Arrhythmias occurred in five patients in the control group and consisted of occasional, isolated ventricular extrasystoles (Lown grade I). Arrhythmias occurred in all patients in the study group. Six patients showed occasional ventricular extrasystoles (Lown grade I). In the remaining 15 patients ventricular extrasystoles were frequent (>30 per hour) but because of complex arrythmias, all fell into a higher grade (Lown grades III and IV). Thirteen patients had ventricular tachycardia (Lown grade IVB) and in only two of these was the arrhythmia not sustained beyond 10 beats. In four patients the run lasted for at least 30 beats. In all instances ventricular tachycardia was self-limiting. The mean arterial pressure in the patients with ventricular tachycardia was significantly higher than that in the remaining patients ($p = 0.0003$) (Fig. 1). All three patients

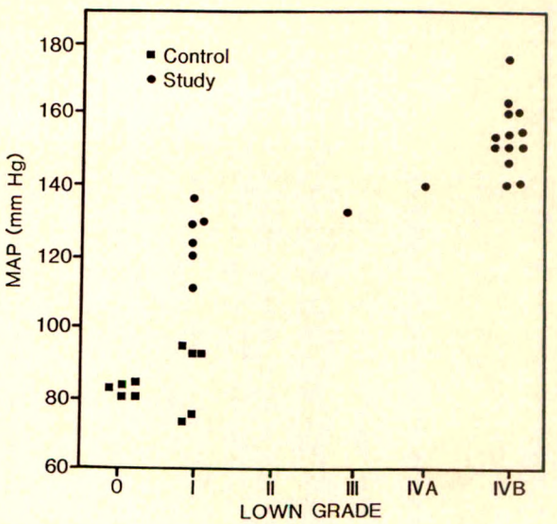


Fig. 1. Grade of arrhythmia versus mean arterial pressure (*MAP*).

with pulmonary edema exhibited ventricular tachycardia on the electrocardiogram (Fig. 2).

Both the incidence of premature ventricular contractions and the Lown grade fell during the delivery phase in 15 patients (71%) and rose immediately thereafter (Figs. 3 and 4). Fourteen of these patients were delivered of infants by cesarean section after general anesthesia was induced. Four of the five patients who were delivered vaginally had high grade arrhythmias (Lown grade IV) that did not abate during delivery. In all patients arrhythmias eventually abated when blood pressure normalized (8 to 12 hours after uterine evacuation).

Comment

This study shows that malignant ventricular arrhythmias occured in 15 of 21 patients with hypertensive crises of pregnancy (71%). These arrhythmias were not related to electrolyte imbalance, deranged acid-base status, or hypoxia. Pulmonary congestion and edema developed in three of the 15 patients with malignant arrhythmias despite a normal precordial assessment. The development of pulmonary edema identifies a high-risk subset of patients and is associated with increased maternal morbidity and mortality.[2]

Several mechanisms have been postulated to explain the pathogenesis of pulmonary edema in these patients including hypervolemia[6] (fluid infusion), pulmonary capillary leakage,[6, 7] and left ventricular failure.[6] Hemodynamic studies have documented raised levels of cardiac output with no evidence of myocardial depression.[8, 9] However, the generalized arterial vasospasm

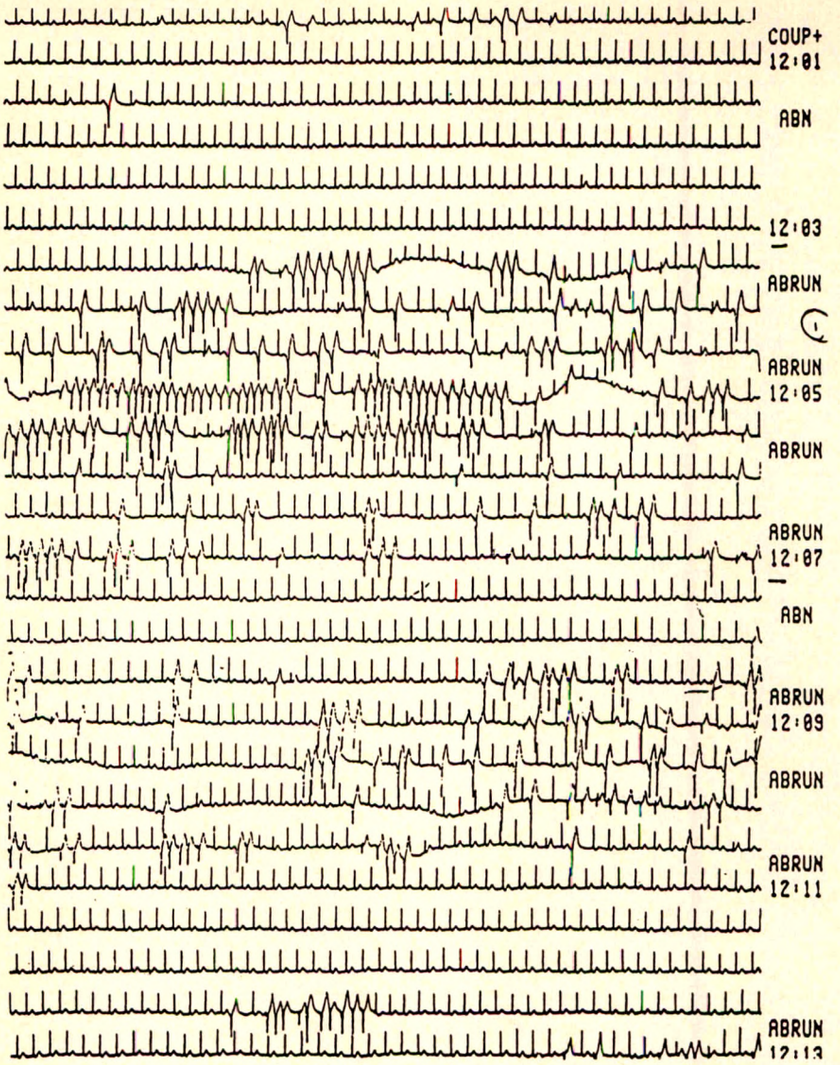


Fig. 2. Sample strip of electrocardiogram with ventricular tachycardia (Lown grade IVB). Patient had pulmonary edema.

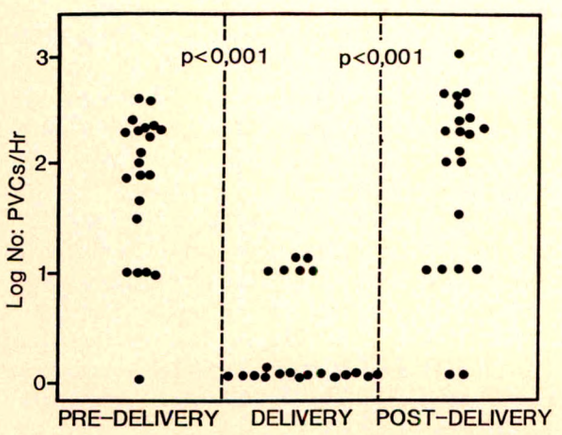


Fig. 3. Frequency of premature ventricular contractions *(PVCs)* per hour in different periodsof delivery.

that is known to be present results in markedly elevated systemic resistance with an increase in stroke work and thereby transient left ventricular dysfunction.[10] In the setting of increased afterload the high frequency of ventricular tachycardia noted in this series provides a more plausible explanation of the development of pulmonary edema and sudden death in these patients.

It appears that a relationship exists between the severity of hypertension and the presence of arrhythmia in patients with hypertensive crises of pregnancy. During the intrapartum phase there was a decrease not only in the frequency of premature ventricular contractions but also the grade of arrhythmia. This was attributed to the mode of delivery. Most of the patients were delivered of infants by cesarean section after induction of general anesthesia. During this period oxygen was administered, blood pressure control was op-

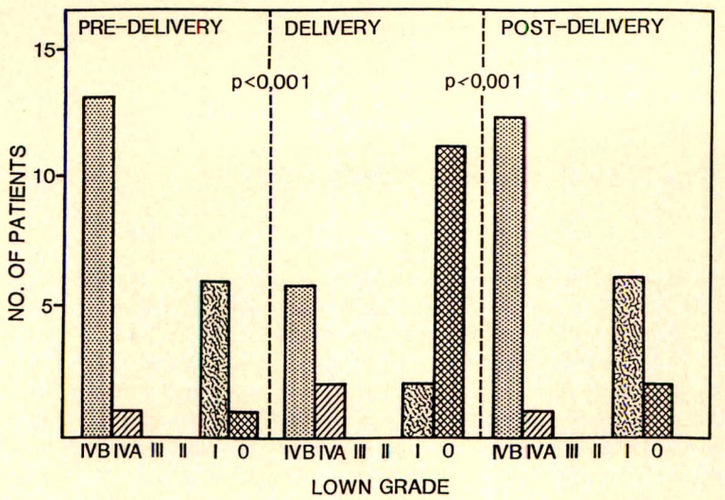


Fig. 4. Grade of arrhythmia in different periods of delivery.

timal, and lidocaine (1 mg/kg) was administered during induction to suppress the hypertensive response to intubation. All three factors probably contributed significantly to control of the arrhythmia. In the five patients who were delivered vaginally lidocaine was not used and so may explain the fact that in these patients the arrhythmias persisted during the intrapartum phase.

The pathogenesis of these arrhythmias is uncertain. Increased ventricular ectopic activity has been seen in patients with essential hypertension when compared with normotensive subjects. However, ventricular arrhythmias were noted to be more prevalent in patients with left ventricular hypertrophy.[11, 12] This does not appear to be the case in patients with hypertensive crises of pregnancy in whom the hypertension was acute and transient. Previous studies showed raised levels of catecholamines in maternal blood collected during labor and after delivery in pregnancies complicated by severe preeclampsia.[13] This may contribute to myocardial necrosis and ventricular irritability. With the generalized vasospasm that occurs in eclampsia, coronary perfusion may also be compromised.[14] Thus the outpouring of catecholamines with resultant tachycardia and elevated systemic vascular resistance could contribute to increased left ventricular stroke work and myocardial ischemia.

This study has practical implications in the management of hypertensive crises of pregnancy. The use of agents that lower blood pressure together with antiarrhythmic properties may be beneficial in the prevention of pulmonary edema and sudden death. The role of β-blockers, the safety of which has been attested in pregnancy.[15] needs to be explored in future studies.

In conclusion, complex ventricular arrhythmias are common in patients with hypertensive crises of pregnancy. This could be a contributory factor in the pathogenesis of acute pulmonary edema and sudden death in these patients.

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Umbilical artery blood flow velocity waveforms during variable decelerations of the fetal heart rate

Erich Weiss, MD, Thomas Hitschold, MD, and Peter Berle, MD
Wiesbaden, Federal Republic of Germany

Blood flow velocities of the umbilical arteries were measured by Doppler ultrasonography during variable decelerations of the fetal heart rate. The flow velocity waveforms, being normal between uterine contractions, showed either an unchanged flow velocity waveform with an exclusive fetal heart rate effect on end-diastolic velocities or a rapid change to absent and reverse diastolic flow during the decelerations, indicating an abrupt increase in placental resistance with a halt in placental perfusion. Computer-aided reconstruction of the fetal heart rate curve revealed the exact temporal relationship between the reduction of umbilical artery perfusion and deceleration of fetal heart rate. We showed that variable decelerations of fetal heart rate can be observed during only slightly changed umbilical perfusion or can be caused by a halt in placental perfusion, which does not necessarily mean an absence of any movement of the fetal blood column, but is a result of a systolic forward and diastolic reverse flow to the same extent. (Am J Obstet Gynecol 1991;164:534-40.)

Key words: Doppler ultrasonography, variable decelerations, umbilical cord occlusion

Doppler ultrasonographic examinations of fetal and maternal vessels are of increasing importance in the antenatal diagnosis of fetal well being and in the diagnosis of placental insufficiency.[1-5] Changes in umbilical artery waveforms can be explained by the fact that changes in placental downstream resistance are caused by histomorphologic alterations of the fetal placenta.[6,7,8] Thus alterations of the umbilical artery waveform without uterine contractions describe a chronic state of the placenta rather than acute parameters of the fetal condition. However, it is interesting that the far more acute changes of flow patterns that might occur in labor have been examined so far by only a few authors. During normal labor the blood flow curves of the fetal aorta descendens[9] are unchanged. The umbilical artery flow velocity waveform shows no significant changes in the systolic/end-diastolic (S/D) ratio throughout normal labor as well.[10,11] We could find only a few publications on Doppler flow measurements of the umbilical artery during typical variable decelerations of the fetal heart rate (FHR) in human beings.[12,13] We thought such an examination allowed an interesting insight into the mechanisms of FHR alterations, which are thought to be caused by flow changes during umbilical cord compression.[14,15]

From the Department of Obstetrics and Gynecology, HSK-Klinikum Academic Hospital of the Johannes Gutenberg University, Mainz.
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Reprint requests: Erich Weiss, MD, Department of Obstetrics and Gynecology, HSK-Klinikum, Ludwig-Erhard-Str. 100, Wiesbaden, Federal Republic of Germany 6200.
6/1/25745

Patients and methods

Eight gravid women were examined during the first stage of labor. In all cases the membranes had ruptured spontaneously or an amniotomy had been performed. The cervix was dilated from 2 to 7 cm. All patients had epidural induction of anesthesia. In all patients regular contractions were present, and cardiotocographic monitoring was carried out continuously by means of an external tocotransducer and a fetal scalp electrode (Hewlett Packard model 8040, HP, Boeblingen, Federal Republic of Germany).

Doppler signals from the umbilical arteries were recorded with a pulsed-wave Doppler system with a 3 MHz duplex transducer (UltraMark 4, Advanced Technological Laboratories, Bothell, Washington). During the examination the patients were in the left lateral decubitus position with head and chest slightly elevated. Fetal movement and fetal breathing were absent during the flow recordings. A 100 Hz thump filter was used routinely and the sample volume was positioned on one umbilical artery identified by real-time ultrasonography.

The technical problems of the flow measurements consisted of a lack of sufficient amniotic fluid and breathing movements of the pregnant woman's abdominal wall. This often led to a temporary loss of Doppler signal during the contractions. The Doppler signal was lost for >10 seconds in 45 of 130 measured contractions and in 50 instances the interruptions showed periods

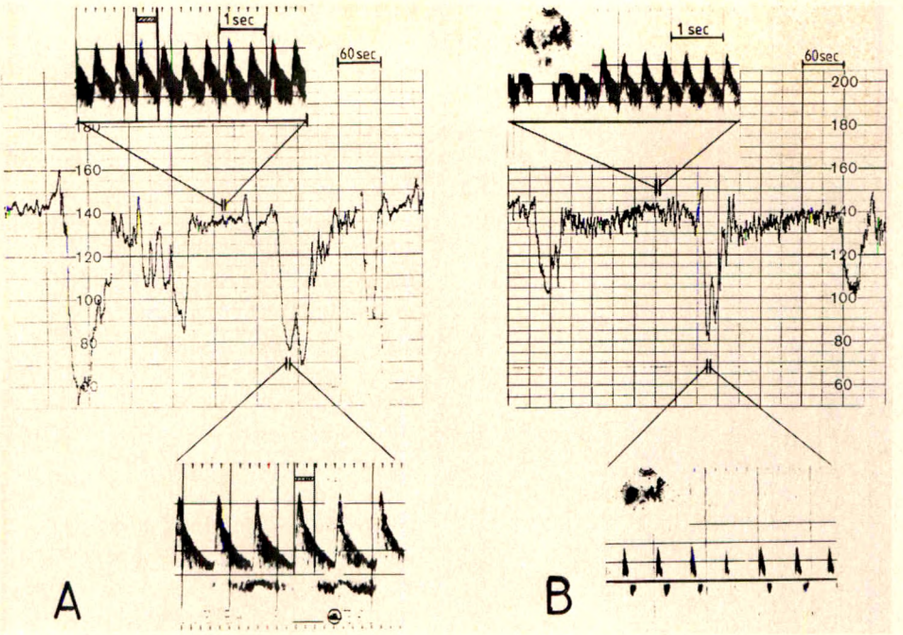


Fig. 1. Flow velocity waveform of umbilical artery before and during typical variable decelerations with FHR effect on flow velocity waveform **(A)** and reverse flow **(B)** during deceleration of FHR.

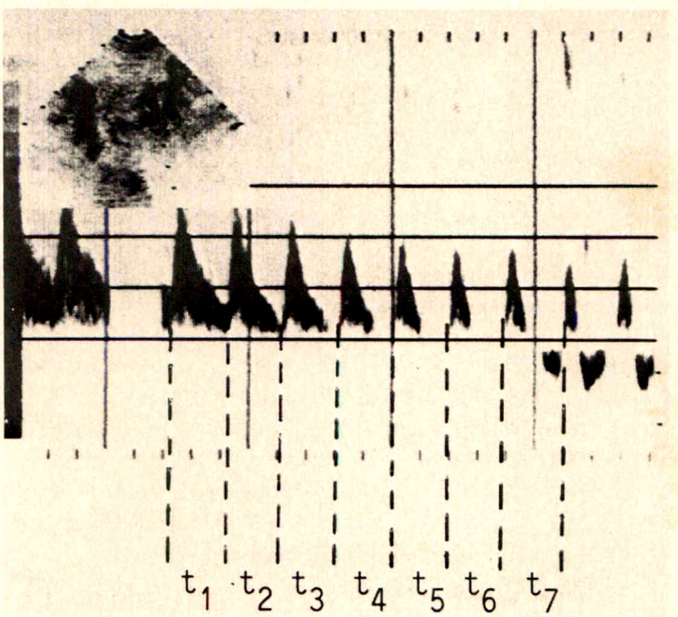


Fig. 2. Length of cardiac cycles on a flow recording during transition from normal flow velocity waveform to diastolic reverse flow = t_x.

of 5 to 10 seconds. Nevertheless acceptable recordings were achieved with a loss of the Doppler signal of <5 seconds during 35 contractions and we could register 22 of them as continuous recordings of the fetal umbilical blood flow during the complete duration of a deceleration. A minimum of two continuous recordings was possible for each fetus.

The flow measurements were continuously recorded

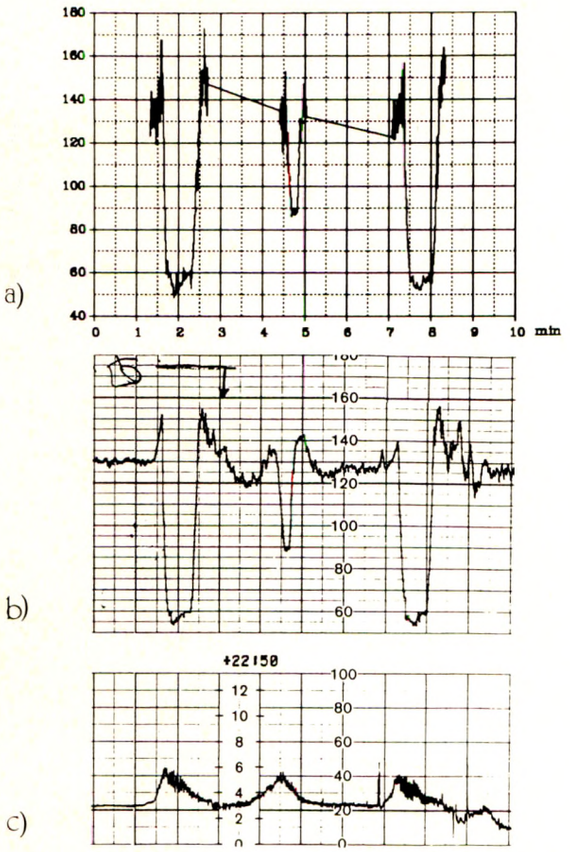


Fig. 3, a. Reconstruction of FHR curve by means of beat-to-beat measurement of Doppler flow velocity waveform; **b,** original FHR-tracing registered by means of scalp electrode; **c,** uterine activity by means of external tocotransducer.

on a video tape recorder. To analyze the flow velocity waveform, we used an IBM-AT personal computer and digitized the video images on a video frame grabber (PCVISION plus, Imaging Technology Inc., Woburn, Mass.). For measurements, a video analysis software was used (JAVA, Jandel Scientifics, Corte Madera, Calif.).

All patients gave informed consent to the study.

Results

Eight fetuses were examined by pulsed Doppler flow measurements of the umbilical arteries during the first stage of labor (39 to 41 weeks' gestation). Samples of fetal scalp blood showed normal pH values in all fetuses except one preacidotic value. Between contractions the blood flow curves showed S/D ratios between 2.0 and 2.5, which is in the normal range (10th to 90th percentile) of our own normal curve.

Although the FHR tracings showed typical variable decelerations in all cases, we recorded two completely different types of flow changes during uterine contractions with variable decelerations. Two fetuses showed a reduction of the diastolic flow velocities that can be exclusively attributed to an FHR effect (Fig. 1, *A*). The elongated heart cycle during the slowed down heart rate allows a decrease of end-diastolic frequency shifts to lower values. However, the pattern of the flow curves was not altered significantly and a measurement of diastolic velocities at a time interval that corresponds to an unchanged FHR reveals an unchanged S/D ratio. In contrast to this, the flow curves in the remaining six fetuses showed a rapid and marked reduction of S/D ratios and a diastolic reverse flow (Fig. 1, *B*). It is evident that an altered FHR is not responsible for the loss of end-diastolic velocities and, in particular, a diastolic reverse flow could not be explained by an extension of the diastolic length of the flow velocity waveform as a result of a decrease in FHR.

To analyze the exact temporal relationship between the decrease of the FHR during variable decelerations and the changes of the flow patterns, we measured the length of each cardiac cycle in the registered Doppler flow curves and calculated the beat-to-beat frequency for each cardiac cycle as it is shown in(Fig. 2). With commercial graphics software we could reconstruct the FHR curve. The result of such a reconstruction is shown in Fig. 3. The calculated FHR curve corresponds to the FHR tracing recorded by means of a scalp electrode.

The extension of the FHR curve to the paper feed velocity of the registered Doppler flow curves was now possible and thus an exact synchronization of FHR-curve and flow pattern was guaranteed.

In Fig. 4 the FHR is plotted simultaneously under the flow pattern, which shows a change to a diastolic reverse flow during a variable deceleration. It is obvious that the change to a diastolic reverse flow occurs 3 seconds before a slight acceleration and 9 seconds before the beginning of the deceleration of the FHR. A similar time delay could be seen in all examined fetuses and varied between a range of 4 to 10 seconds between the beginning of reverse flow and the beginning of the deceleration of the FHR. Furthermore, Fig. 4 shows that during the second part of the w-shaped deceleration, diastolic flow velocities were registered in the normal range again when the FHR was still below 100 beats/min.

To be able to estimate the extent of the flow reduction during the described changes in flow patterns, we measured the area under the flow curve in each cardiac cycle by use of the described video analysis system. The use of planimetry presumes that the flow velocities under the curve are distributed proportionally. In particular, during forward flow this leads to a slight underestimation of the flow inasmuch as there is a discontinuous distribution of the flow velocities with a higher density at the top margin of the curves. Nevertheless this is a sufficiently exact method to estimate the size

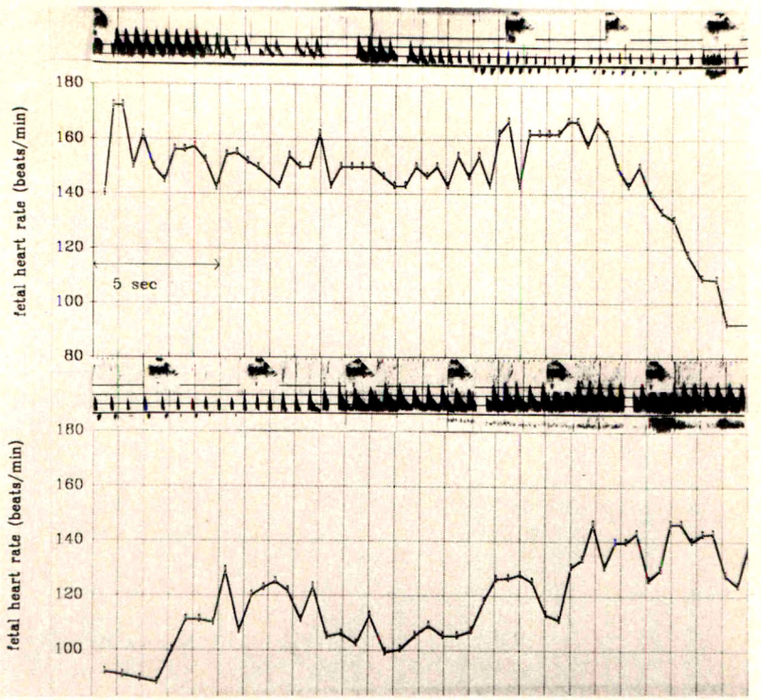


Fig. 4. Synchronized Doppler flow velocity waveform and reconstructed FHR tracing during variable deceleration with reverse flow.

of the flow. During deceleration of the FHR the length of each cardiac cycle is extended and therefore in cases of an unchanged flow velocity waveform the estimated flow per cardiac cycle increases. For that reason we calculated the flow per cardiac cycle and per minute. Setting the flow curve between the contractions in each case to 100%, we plotted the relative decrease in perfusion against the FHR. In Fig. 5 this is done for the deceleration shown in Fig. 4. There is a sharp decrease in umbilical perfusion before the FHR decreases. During the reverse flow the area under the curve in the forward flow channel is equal to that measured in the reverse flow channel and this corresponds to a movement of the blood column forward and backward to about the same extent. The umbilical perfusion reached normal values about 6 seconds before the FHR reached a normal baseline rate.

Fig. 6 shows the continuous registration of the Doppler signal in a case of an FHR-altered flow velocity waveform during a variable deceleration. The perfusion during this deceleration expressed as relative umbilical artery blood flow per minute is reduced simultaneously to the decelerating FHR (Fig. 7). However, whereas the FHR decelerates to about 50% of the normal baseline, the relative umbilical artery blood flow is reduced by only 30%. This is because of an increase of the area under the flow curve caused by the prolonged heart cycle and the unchanged flow velocity waveform during

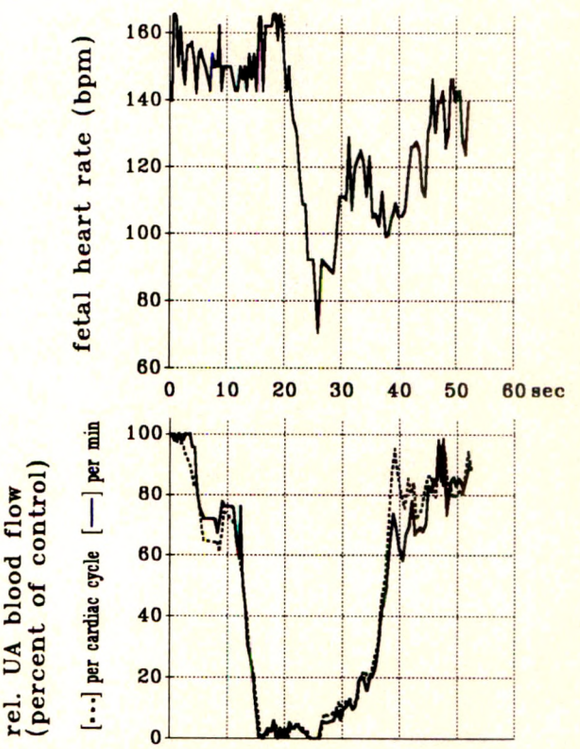


Fig. 5. Reconstructed FHR-tracing *(top)* and relative perfusion of umbilical artery during variable deceleration with reverse flow *(bottom)*.

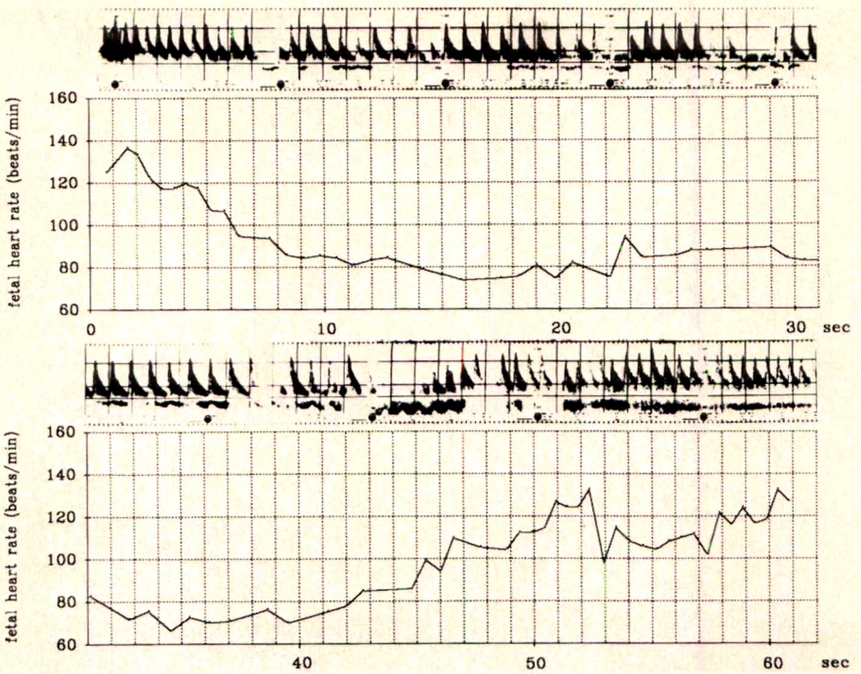


Fig. 6. Synchronized Doppler flow velocity waveform and reconstructed FHR tracing during variable deceleration with FHR effect on flow velocity waveform.

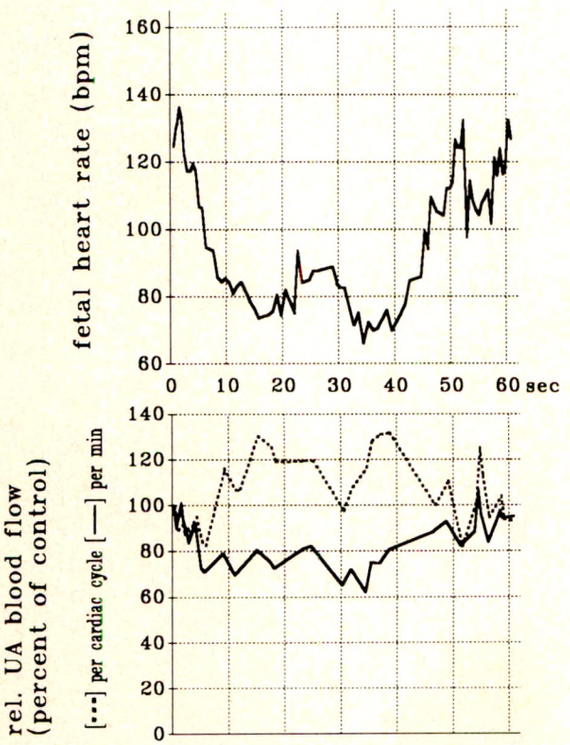


Fig. 7. Reconstructed FHR tracing *(top)* and relative perfusion of umbilical artery during variable deceleration with FHR effect on flow velocity waveform *(bottom)*.

the deceleration of the FHR. Therefore the calculated flow volume per heart cycle increases parallel to the decrease of the FHR, leading to only a slight reduction of perfusion per minute.

Comment

The effects of a reduction of the umbilical perfusion on fetal blood circulation were examined by several authors in sheep fetuses.[16, 17] Künzel et al.[17] could show that the reduction of umbilical vein perfusion by partial cord compression results in a marked increase of the umbilical vein blood pressure. Inasmuch as the systolic and diastolic blood pressure in the fetal aorta was only slightly elevated during umbilical vein occlusion, this led to a reduction of the perfusion pressure of the placenta. During systole the effective perfusion pressure was 75% and during diastole only 30% of control values. The direct evidence of the linear relationship between increase of umbilical vein pressure and decrease of the flow in the umbilical arteries was performed by Berman et al.[16] Furthermore, these authors showed the linear correlation between the mean arterial blood pressure and the blood flow in the umbilical arteries.

In our study we confirmed these experimental results also in the human fetus with the noninvasive Doppler method. In instances in which a significant reduction of umbilical perfusion is present, the recordings of the

flow profiles (in contrast with the overall measurements of blood flow with electromagnetic flow probes) allow an investigation of the mechanism of flow reduction in the umbilical arteries. We found that the reduction of perfusion is caused by a significant decrease of systolic velocities and by a halt in or reversal of flow of the blood column during diastole. Inasmuch as the perfusion pressure, that is the difference between aortic blood pressure and umbilical vein blood pressure, causes the forward flow through the placental vessels, we conclude that this arteriovenous pressure gradient decreased during the measured variable decelerations to diastolic values that were too low to cause forward flow through the placenta throughout the diastole. If the diastolic umbilical vein blood pressure is as high as the diastolic aortic blood pressure of the fetus, the diastolic venous outflow of the placenta decreases to zero. This situation is given in cases of a nearly total occlusion of the umbilical vein in which only the systolic arteriovenous pressure gradient is high enough to cause a venous outflow of the placenta. This status is present for some seconds during the transition from normal to reverse flow and vice versa (Fig. 4).

However, the total occlusion of the umbilical vein allows no venous passage, and therefore the stroke volume that is moved by the high systolic blood pressure in the fetal aorta into the arterial tree of the placenta, leads to a high diastolic pressure in the placental arteries, inasmuch as their compliance represents a second compression chamber. If its pressure exceeds the diastolic pressure in the aorta, a diastolic reverse flow from the arterial vascular tree of the placenta to the fetal aorta results.

This hypothesis induces that the complete occlusion of the fetal umbilical vein does not lead to a stop of the blood column in the umbilical arteries as could be assumed by the studies mentioned with electromagnetic flow measurement. On the contrary we found a highly pulsatile flow pattern in the umbilical arteries during cord compression. In our studies we showed that this causes a temporary ineffective perfusion by moving the blood column forward and backward to the same extent.

The continuous measurement of the umbilical artery flow velocity waveform by Doppler ultrasonography during variable decelerations of the FHR proves that two primary mechanisms exist that cannot be differentiated by analysis of the FHR tracings alone. The first one is discussed above and is in good agreement with the generally accepted opinion[14, 15] that variable decelerations are caused by a temporary mechanical occlusion of the umbilical perfusion. We showed that the decrease in effective flow in the umbilical artery to zero occurs at a time when the FHR does not yet show any alteration. The time interval between this initial event and the deceleration of FHR is in good agreement with the lag found in sheep after umbilical vein occlusion.[17] However, in some cases, variable decelerations of the FHR are obviously not caused by a mechanical reduction of the umbilical perfusion. Our results show that the only effect on umbilical perfusion in this case is a prolonged diastolic decrease of flow velocities as a result of the decelerated FHR. The reduction of the relative perfusion per minute is only moderate and is in sharp contrast to the marked deceleration of the FHR (Fig. 6). We conclude that this is a parasympathetic reaction of the central nervous system caused by a local or systemic hypoxemia of the fetus. Therefore Doppler examinations of the umbilical arteries during labor could help to differentiate whether decelerations of the FHR are caused by hypoxic reasons or by mechanical occlusion of the umbilical vein and subsequently by a halt in arterial perfusion. Further investigation is necessary to confirm the clinical value of our results.

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A case report of giant cell arteritis of the uterus and adnexa

P.R. Summers, MD,[a] M.K. Biswas, MD,[a] D.W. Boulware, MD,[b] L. Green, MD,[c] E.H. Herrera, MD,[a] and A.G. O'Quinn, MD[c]

New Orleans, Louisiana

Giant cell arteritis of the uterus and adnexa is a rare disorder, which is generally asymptomatic and occasionally associated with temporal arteritis. The true clinical significance of this dramatic microscopic finding remains obscure, but arteritis of the uterus and adnexa may be associated with later development of giant cell arteritis elsewhere in the body. (Am J Obstet Gynecol 1991;164:540-2.)

Key words: Giant cell arteritis of uterus, pelvic vasculitis, temporal arteritis

The following case report concerns the appearance of giant cell arteritis in the uterus of a 68-year-old patient with temporal arteritis. Although giant cell arteritis of the uterus and adnexa is rare, doctors who are treating women with temporal arteritis should be alerted to the possibility for this associated condition, which may be asymptomatic.

Case report

A 68-year-old woman, gravida 1, para 0, abortus 1, was seen as an outpatient for routine gynecologic evaluation. Her only complaint was epigastric burning at night with mild episodic abdominal bloating; this was considered to be related to prednisone therapy for temporal arteritis, which was diagnosed 6 months earlier (Fig. 1). Palpation of an asymptomatic adnexal mass led to a pelvic ultrasonographic study that demonstrated a left ovarian cyst of 2.5 × 2.9 cm in size. At this point the patient refused surgery. A new finding of septation of the cyst was noted on repeat ultrasonographic evaluation 4 months later, although size of the cyst was unchanged. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. Pathologic evaluation of the surgical specimen demonstrated a serous cystadenoma of the left ovary with extensive granulomatous arteritis identified throughout the uterus, fallopian tubes, and ovaries (see Fig. 2). The patient's recovery from surgery was uneventful. Temporal arteritis was in remission, and she has not been treated with prednisone for several months now. She declined further steroid therapy for presumed persisting but asymptomatic pelvic arteritis.

Comment

Giant cell arteritis of the uterus and adnexa is indeed a rare finding. A review of the world literature reveals only 15 reported cases of this disorder that affects the uterus.[1] In two of these reported cases the patient was also noted to have temporal arteritis. The number of the remaining patients in whom symptomatic temporal arteritis eventually developed is not reported. No case of giant cell arteritis that involved other areas of the body could be found in the literature that is associated with this disorder of the uterus and adnexa. One would presume, though, that such an association with vascular disease in other organs would be likely, since giant cell arteritis is considered to be a disease with the potential for systemic involvement.[2]

One would assume that the severity of symptoms

From the Departments of Obstetrics and Gynecology,[a] Medicine,[b] and Pathology,[c] Tulane University School of Medicine.
Received for publication April 24, 1990; revised August 2, 1990; accepted August 22, 1990.
Reprint requests: Paul R. Summers, MD, Department of Obstetrics and Gynecology, Tulane University School of Medicine, 1430 Tulane Ave., New Orleans, LA 70112.
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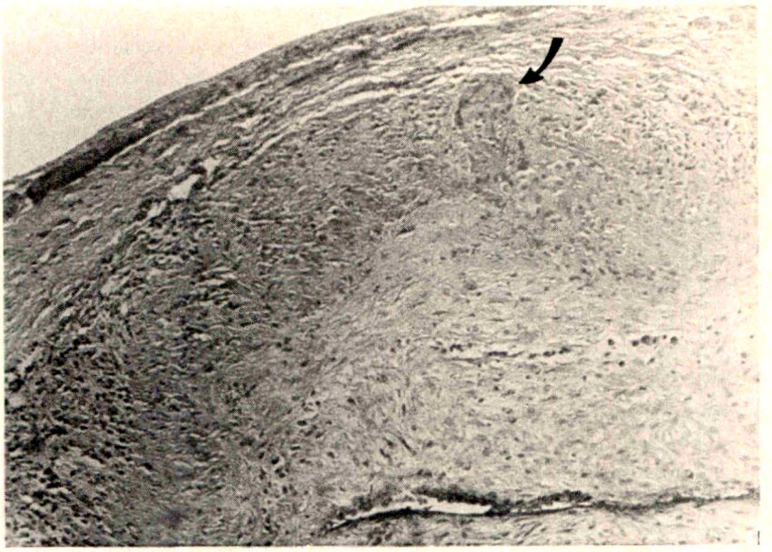


Fig. 1. Longitudinal section of temporal artery, that shows nodular thickening of vascular wall, slitlike appearance of vascular lumen, and near occlusion of the lumen. The tunica media is infiltrated by chronic inflammatory cells and histiocytes, with the formation of multinucleated giant cells *(arrow)*. (Hematoxylin-eosin stain; ×400.)

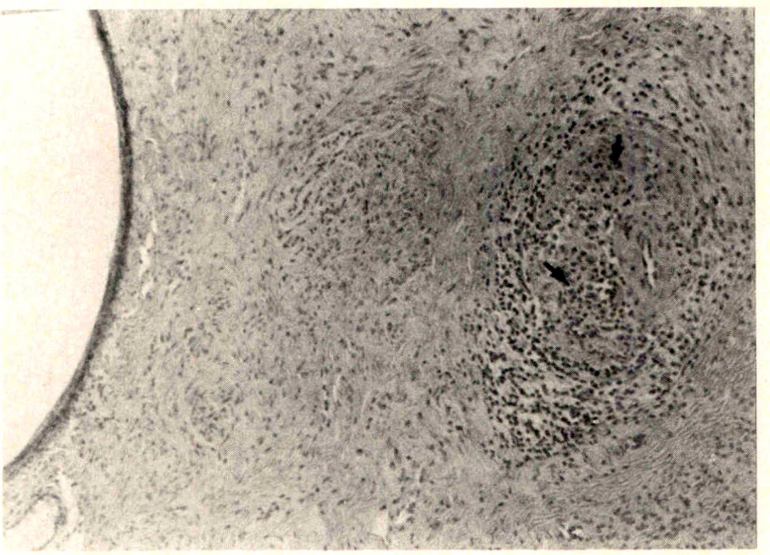


Fig. 2. Section of ovary. In this instance the inflammatory infiltrate within the vessel wall consists largely of chronic inflammatory cells; however, clusters of histiocytes are seen *(arrows)*. Note the markedly narrowed vascular lumen. (Hematoxylin-eosin stain; ×400.)

from giant cell arteritis would directly relate to the severity of organ involvement. A review of the literature review reveals no cases of symptomatic giant cell arteritis of the uterus. This is consistent with the asymptomatic status of the patient in this report. Her lack of symptoms may be related to the postmenopausal state with relative inactivity of the ovaries and endometrium. Yet it is surprising that this patient was totally free of symptoms, in view of the extensive vasculitis with multiple areas of arterial occlusion, which was seen microscopically throughout the surgical specimen.

Therapy for giant cell arteritis, a disorder of unclear

causes, is generally successful with a course of prednisone. It is surprising that this patient's previously symptomatic temporal arteritis remained clinically in remission during the episode of active pelvic vasculitis. One would presume that residual tissue in the pelvis after the patient's hysterectomy would continue to be involved with this inflammatory process, considering the extent of vasculitis in the surgical specimen. It is difficult to assess the significance of such residual disease after hysterectomy. This patient declined further steroid therapy for the detected pelvic vasculitis because of the unpleasant side effects she had previously experienced.

In summary, the true clinical significance of isolated giant cell arteritis of the uterus and adnexa in a postmenopausal patient remains obscure. In such a case, though, it would be logical to maintain surveillance for development of giant cell arteritis at other body sites. Careful physical examination and repeated evaluation of erythrocyte sedimentation rate would be important. Giant cell arteritis of the pelvis can occur, and physicians who are treating temporal arteritis should be aware of the possibility of association giant cell arteritis of the uterus and adnexa. It will be of interest if future similar case reports reveal associated menstrual disorders in premenopausal patients. Giant cell arteritis that is found coincidentally during evaluation of a hysterectomy specimen should warrant a search for temporal arteritis. Furthermore, the recommendation for treatment of asymptomatic giant cell arteritis of the pelvis is unclear, since this patient's untreated arteritis is thus far benign after more than 12 months of close follow-up.

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Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility

R.J. Aitken, PhD,[a] D.S. Irvine, MD,[b] and F.C. Wu, MD[a]
Edinburgh, Scotland

We have undertaken a prospective analysis of the diagnostic significance of three different criteria of human sperm function including the conventional semen profile, measurements of hamster-oocyte fusion, and determinations of reactive oxygen species generation in 139 couples. The latter, who were characterized by a lack of detectable abnormalities in the female partner, were followed up for a maximum of 4 years to determine the incidence of spontaneous pregnancy in the absence of therapeutic intervention. Assessments of monthly fecundity with life table analysis techniques revealed a highly significant, positive relationship between fertility and hamster-oocyte fusion rates that were measured in the presence of the ionophore, A23187. Conversely, reactive oxygen species generation was shown to be negatively associated with both the outcome of the sperm-oocyte fusion assay and fertility in vivo. The clinical significance of these diagnostic techniques was emphasized by the fact that within the same data set, the conventional semen profile was of no significant diagnostic value. (Am J Obstet Gynecol 1991;164:542-51.)

Key words: Sperm-oocyte fusion, reactive oxygen species generation, fertility

From the Medical Research Council Reproductive Biology Unit[a] and Department of Obstetrics and Gynaecology,[b] Centre for Reproductive Biology.
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Reprint requests: Dr. R. J. Aitken, MRC Reproductive Biology Unit, 37 Chalmers St., Edinburgh, Scotland EH3 9EW.
6/1/25750

Awareness of the deficiencies of the conventional semen profile as the sole means of the assessment of male fertility has led to the development of a new generation of alternative diagnostic tests that are based on a functional analysis of the fertilizing potential of human spermatozoa.[1] Foremost among these is a test intro-

duced by Yanagimachi et al. in 1976,[2] which is based on the unique ability of the zona-free hamster oocyte to fuse with human spermatozoa. The biologic significance of this fusion process is based on the fact that this event can only occur if the spermatozoa have undergone the acrosome reaction and successfully generated a fusogenic plasma membrane. Furthermore, the ultrastructural details of sperm-oocyte fusion in this system appear to be identical to the homologous situation in that fusion with the oolemma is initiated by a discrete area of plasma membrane that overlies the equatorial segment of acrosome-reacted spermatozoa.[3]

As a means of diagnosing male fertility the general consensus of the retrospective studies that addressed this issue is that this procedure has the potential to discriminate between fertile and infertile men, although in practice there has been a general failure to standardize the methods for the test and to introduce appropriate quality control measures.[4, 5] The only two prospective studies[6, 7] that have investigated the long-term prognostic value of the test have tended to confirm the ability of the zona-free hamster-oocyte penetration test to indicate the relative fertility of male patients, although some resultant pregnancies were recorded for subjects who exhibited abnormal penetration test results.

In evaluation of this particular test it must be recognized that: (1) the zona-free hamster oocyte penetration assay is only capable of generating information on one component of sperm function, namely the ability of acrosome-reacted cells to initiate sperm-oocyte fusion and (2) the major biologic defect of the assay is that it is entirely dependent on the occurrence of spontaneous acrosome reactions in populations of human spermatozoa that have been incubated in vitro. This is unsatisfactory because the incidence of spontaneous acrosome reactions in vitro is extremely low (5% to 10%),[8] and the mechanisms by which this event is triggered by prolonged incubation may bear no relationship to the physiologic process as it occurs in vivo.

To address this inherent defect in the sperm penetration assay we modified the protocol for this test[9] by including the divalent cation ionophore, A23187, in the preincubation medium. The purpose of the ionophore is to generate those intracellular changes in pH and free ionic calcium that are responsible for initiation of the acrosome reaction,[10] and to this extent, the action of A23187 is physiologic. Under the influence of this reagent, an increase in the proportion of acrosome-reacted cells manifests itself as a highly significant rise in the levels of sperm-oocyte fusion, at least within the normal fertile population.[9] However, in certain cases of male infertility the intracellular signals that are generated by A23187 are not sufficient to stimulate an enhancement in the levels of sperm-oocyte fusion.[9] Such refractoriness to ionophore stimulation suggests that in these patients there are defects in the spermatozoa, downstream from calcium influx, that prevent the plasma membrane from undergoing those fusogenic changes that are normally associated with the acrosome reaction and penetration of the oocyte. Recent studies in this area have suggested that pathologic events, that involve the peroxidation of unsaturated fatty acids in the sperm plasma membrane may be responsible for this refractory state.[11, 12] This peroxidative damage appears to be initiated by the excessive generation of reactive oxygen species by the ejaculate and results in a loss of membrane fluidity and integrity such that the spermatozoa are no longer able to exhibit biologic responses in the presence of ionophore.[11-13]

In view of these results, we undertook a detailed evaluation of reactive oxygen species generation and A23187-induced sperm-oocyte fusion in the diagnosis of human infertility. This evaluation took the form of a prospective study in which the incidence of spontaneous pregnancy was monitored during a median follow-up period of 510 days in 139 untreated couples who were characterized by a normal female partner. We believe that this is a unique data set that sheds new light on the diagnostic potential of these tests.

Patients and methods

Patients. This study was based on a prospective analysis of the incidence of spontaneous pregnancy in 139 couples who were characterized by a normal female partner, in terms of history and examination, menstrual cycle duration and regularity, and the outcome of a diagnostic laparoscopy including a tubal patency test that is based on the passage of methylene blue through the female reproductive tract. The male partners were unselected, apart from the exclusion of azoospermia, and a full description of their semen profiles is given in the Results section. The median duration of infertility at the time of analysis was 60 months with 10th to 90th percentiles of 36 and 120 months.

Study design. A detailed semen analysis was performed for each male partner including the conventional profile, zona-free hamster oocyte penetration test, and assessment of reactive oxygen species generation. The couples were then left untreated for a median follow-up period of 17 months, with 10th to 90th percentiles of 8.3 to 33.3 months, during which time 32 women (23%) spontaneously conceived. An analysis of the relationship between the criteria of semen quality that was measured at the beginning of the study and the subsequent incidence of spontaneous pregnancy was then performed. In this way, a measure of the relative prognostic value of the hamster oocyte penetration test, reactive oxygen species generation, and conventional semen profile was obtained.

Statistics. In view of the abnormal distribution that was exhibited by a majority of the variables, the data are presented as median values with 10th to 90th percentiles, and parameteric forms of analysis such as multivariate discriminant analysis have been avoided. Differences in the distribution of data between groups were assessed with the Mann-Whitney U test, and differences in frequency were evaluated by χ^2 analysis. Where linear regression coefficients have been calculated the data have been transformed ($\sqrt{x}$, log x, log 1 + x) to optimize the normality of the data distribution.

Life table analysis was performed with the procedures described by Cramer et al.,[14] which generated data on the cumulative pregnancy rate and monthly fecundity.

Conventional semen profile. The patients produced semen samples for analysis by masturbation into sterile plastic containers. After at least 30 minutes was allowed for liquefication to occur, a semen profile was constructed with the procedures described by the World Health Organization[15] and included measurement of sperm concentration, total count, percent normal morphology, and percent motility.

Hamster oocyte penetration test. The spermatozoa were washed by repeated cycles of dilution and centrifugation (500 g for 5 minutes) with 5 to 8 ml volumes of medium BWW[16] and finally resuspended at a concentration of 20×10^6 cells/ml. They were then diluted with an equal volume of BWW that contained an aqueous suspension of the calcium magnesium salt of A23187 as described previously.[9] All samples were subjected to a 3-hour exposure to A23187 at a final concentration of 0.025 mg/ml, which we previously showed to be optimal for a majority of specimens.[9] In 33 cases, however, sufficient material was available to carry out an additional assessment of sperm-oocyte fusion at a dose of 0.05 mg/ml. In such cases in which the spermatozoa were examined at two doses of ionophore, the higher result was taken for analysis. After this 3-hour incubation with ionophore, the samples were formed into pellets by centrifugation (500 g for 5 minutes) and resuspended at a concentration of 10×10^6/ml before dispersal as 50 μl droplets under liquid paraffin. Zona-free hamster oocytes were then introduced into the droplets (approximately 20 oocytes per sample) and after a further 3 hours of incubation, they were examined for the presence of decondensing sperm heads by phase-contrast microscopy at a magnification of ×250.

In every test run a quality control sample of known fertilizing capacity was monitored to confirm the validity of the assay in terms of medium composition of oocyte integrity. These control samples gave a mean penetration rate of 74.0% ± 2.9% with an average of 3.0 ± 0.3 spermatozoa fusing with each oocyte (n = 70).

Reactive oxygen species generation. Reactive oxygen species generation was monitored with the chemiluminescent techniques described previously, and luminol (5-amino-2,3-dihydro-1,4 phalazinedione) was used as the probe. In brief, suspensions of human spermatozoa at 20×10^6 cells/ml in a volume of 500 μl were exposed to 1 μl of luminol that was stored as a 100 mmol/L stock solution in dimethyl sulfoxide. Five hundred microliters of either medium BWW (control) or medium BWW that contained A23187 (0.05 mg/ml aqueous suspension of the calcium magnesium salt) was then added, and the chemiluminescence was monitored as counts integrated over a 10-second period after an incubation period of 3 minutes in a Berthold Biolumat, L139500T luminometer (Berthold Analytical Instruments Inc., Nashua, N.H.).

Results

Analysis of the conventional parameters of semen quality for this patient population gave median (10th to 90th percentile) values for sperm concentration and motility and normal morphology of 56 (16.0 to 156.2) $\times 10^6$ cells/ml, 53% (12% to 65%) and 46.5% (21.7% to 64.6%), respectively. Of the 139 patients who were examined in this study, a majority (75) were normospermic according to the criteria that we established for our local fertile population of $>20 \times 10^6$ spermatozoa per milliliter, >40% normal morphology, and >40% motility.[17] Of the remainder, 23 were normal except for isolated mild teratospermia (30% to 40% normal morphology), and in 10 other patients the isolated teratospermia was more severe (<30% normal). Six patients exhibited isolated asthenospermia, and in eight patients motility and morphology were defective (asthenospermia and teratospermia), but the sperm count was normal. In two patients isolated oligospermia was seen and in another two, the oligospermia was associated with asthenospermia. In four patients the low sperm counts were associated with teratospermia and in nine, the complete oligoasthenoteratospermia syndrome was observed.

Reactive oxygen species. The chemiluminescent technique that was used to monitor reactive oxygen species production revealed an extremely wide range of spontaneous activity on the part of the washed sperm preparations. The median basal chemiluminescent signal was 237 counts per 10 seconds and the 10th and 90th percentiles were 88 and 10,034 counts per 10 seconds, respectively. After the addition of ionophore A23187 there was a significant elevation of reactive oxygen species production ($p < 0.001$) to give a median value of 815 counts per 10 seconds and 10th and 90th percentile figures of 144 and 34,056 counts per 10 sec-

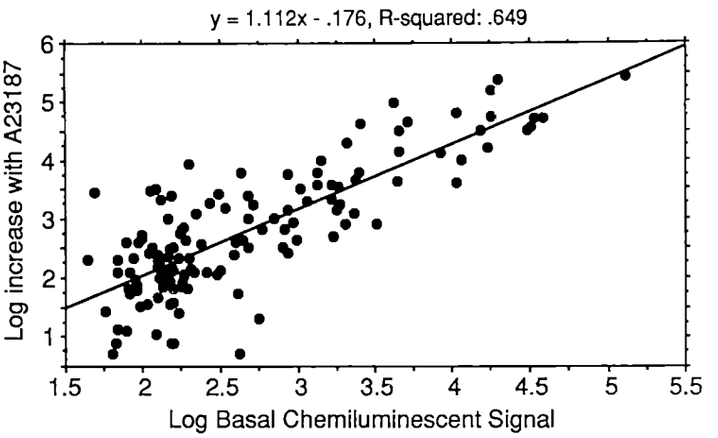


Fig. 1. Relationship between basal, spontaneous level of reactive oxygen species generation and degree of enhancement observed in presence of A23187.

onds. The magnitude of response to A23187 again showed considerable variation from individual to individual; the median difference between the basal-stimulated and A23187-stimulated signals was 341 counts per 10 seconds, and the 10th and 90th percentile values were 9 and 25,942 counts per 10 seconds, respectively. Linear regression analysis following logarithmic transformation of the chemiluminescent data revealed that the magnitude of the cellular response to A23187 was highly correlated ($r = 0.805$; $p < 0.001$) with the basal, spontaneous level of reactive oxygen species generation (Fig. 1).

Zona-free hamster oocyte penetration. In 136 patients the spermatozoa were assessed with the zona-free hamster oocyte penetration test after the induction of the acrosome reaction with A23187. This population of patients exhibited a wide range of functional competence according to this test, which provided a median percentage penetration value of 26.7% (10th to 90th percentiles of 0% and 100%, respectively); corresponding figures for the mean number of spermatozoa that fused with each oocyte were 0.278 and 0 to 4.206.

Relationship between sperm-oocyte fusion and reactive oxygen species generation. Scatterplot analysis of the relationship between reactive oxygen species generation and sperm-oocyte fusion revealed the same negative association between defective sperm function and high basal levels of reactive oxygen species production that we observed in earlier studies (Fig. 2). If, on the basis of published analyses of the normal fertile population,[13] we took the thresholds of normality to be 10,000 counts per 10 seconds before the addition of A23187 and 30,000 counts per 10 seconds at 3 minutes after the addition of ionophore, then seven patients were found to exhibit abnormally high levels of reactive oxygen species generation. Within this subpopulation

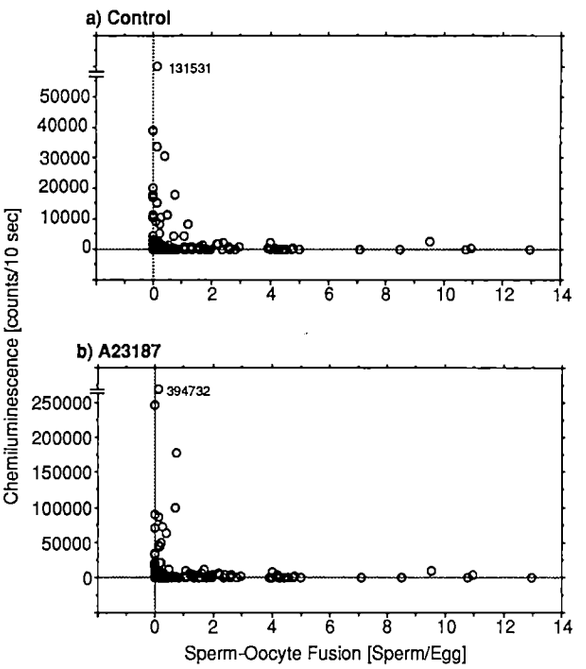


Fig. 2. Scatterplots of relationship between sperm-oocyte fusion and **(a)** control and **(b)** A23187-stimulated levels of reactive oxygen species generation.

of patients the spermatozoa exhibited a median (10th to 90th percentile) of only 10% (0% to 45.6%) oocyte penetration and 0.1 (0 to 0.6) sperm fusions per oocyte. This level of sperm-oocyte fusion was significantly ($p = 0.0026$) lower than that in the remainder of the study population.

Relationships with pregnancy

Luminol signal. Analysis of the relationship between reactive oxygen species generation and spontaneous pregnancy rate revealed a clear negative association as

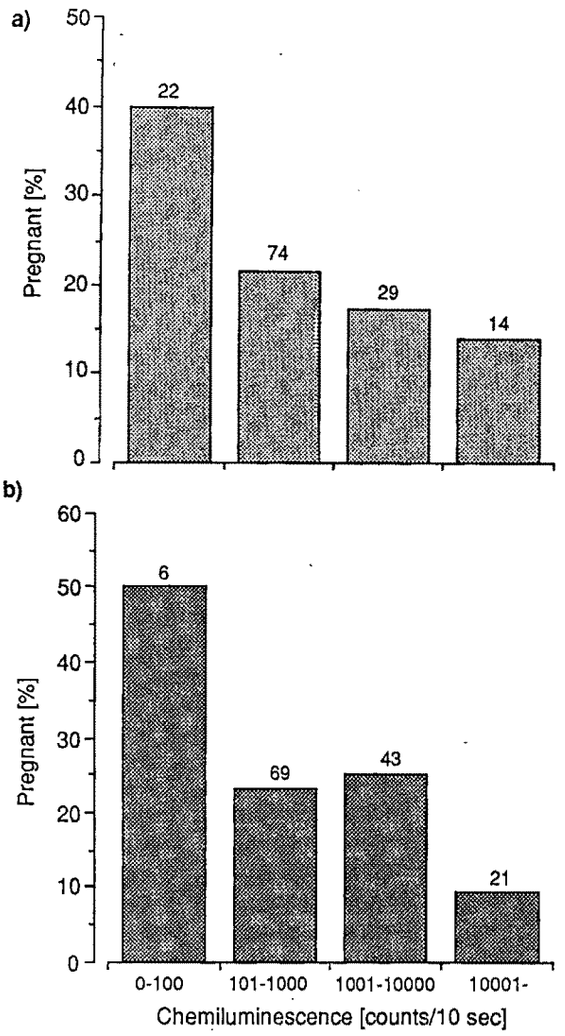


Fig. 3. Incidence of spontaneous pregnancy in relation to levels of reactive oxygen species generation observed in **(a)** absence and **(b)** presence of ionophore, A23187.

illustrated in Fig. 3. Analysis of the basal chemiluminescent signal, which was obtained in the absence of A23187 (Fig. 3, *a*), revealed the presence of a cohort of highly fertile patients ($n = 22$) who exhibited very low levels of reactive oxygen species production (chemiluminescent counts <100 counts per 10 seconds) in association with a high pregnancy rate of 40%. When the basal chemiluminescent counts increased to between 101 and 10,000 counts per 10 seconds, the pregnancy rate was halved to around 20%, and with basal luminol signals of >10,000 counts, a pregnancy rate of 14% was recorded.

After the addition of A23187 the chemiluminescent signals were significantly elevated ($p < 0.001$). However, six patients still exhibited luminol counts of <100 counts per 10 sec, and three (50%) of them spontaneously achieved pregnancy. With chemiluminescent counts of 100 to 10,000 the pregnancy rate remained at around 20%, and with counts above 10,000 only 9% (2 of 21) of the patients initiated a pregnancy; none of the four male patients who exhibited luminol signals of >100,000 counts per 10 seconds was successful in this regard (Fig. 3, *b*).

Table I. Monthly fecundity rates for different levels of spontaneous reactive oxygen species generation

Chemiluminescent (counts/10 sec)	*No. of patients*	*Monthly fecundity*	*95% Confidence intervals**
0-100	22	0.012	0.0042-0.02
101-1000	74	0.0057	0.0029-0.0084
1001-10,000	29	0.0045	0.0006-0.0085
>10,000	14	0.0033	0-0.0084

*Two-sided.

Life table analysis of cumulative pregnancy rates against basal levels of reactive oxygen species production gave monthly fecundity values that ranged from 0.012 to 0.0033 for patients at the extremes of the data distribution (Table I). Comparison of the monthly fecundity values for each stratum of the reactive oxygen species data set, with the Z statistic described by Cramer et al.,[14] revealed significant differences ($p < 0.05$) in the monthly fecundity rates between the group that exhibited the lowest chemiluminescent count of 0 to 100 counts per 10 seconds and the monthly fecundity values that were recorded for all the other levels of reactive oxygen species generation (Fig. 4, *a*).

Life table analysis also indicated a decline in monthly fecundity values with increasing levels of reactive oxygen species generation in response to A23187 (Fig. 4, *b;* Table II). The particularly low monthly fecundity value that was obtained for patients who exhibited high chemiluminescence responses (more than 10,000 counts per 10 seconds) after stimulation with A23187 (monthly fecundity = 0.0023) was significantly lower than the monthly fecundity values that were recorded for the patients who exhibited reactive oxygen species generation in the ranges of 0 to 100 and 101 to 1000 counts per 10 seconds. Conversely, the relatively high monthly fecundity value (0.016) that was recorded for the small group of patients who exhibited the lowest (less than 100 counts per 10 seconds) level of reactive oxygen species generation was significantly ($p < 0.05$) greater than the monthly fecundity values that were recorded for all the higher levels of activity.

Thus the most telling components of the reactive oxygen species data set appeared to be at the extremes of the frequency distribution rather than at the center, where the median values (10th to 90th percentile) for the pregnant and nonpregnant cohorts of patients were

Table II. Monthly fecundity rates for different levels of reactive oxygen species generation after A23187

Chemiluminescent (counts/10 sec)	*No. of patients*	*Monthly fecundity*	*95% Confidence intervals**
0-100	6	0.016	0.0022-0.035
101-1000	69	0.0061	0.0031-0.0092
1001-10,000	43	0.0069	0.0028-0.01
>10,000	21	0.0023	0.0009-0.0055

*Two-sided.

not significantly different at 181 (75 to 4645) and 271 (941 to 10,700) counts per 10 seconds in the absence of A23107 and 518 (116 to 9301) and 875 (157 to 42,010) in the presence of this reagent.

Hamster oocyte fusion. An analysis of the incidence of pregnancy against hamster oocyte penetration rate is presented in Fig. 5. The results reveal the existence of a clear positive relationship between this criterion of sperm function and fertility, regardless of whether sperm per oocyte or percentage penetration is used as the end point for the assay. A false-negative pregnancy rate of 4.1% (1 of 24) was recorded for the patients who exhibited 0% penetration in the hamster oocyte assay. Thereafter the pregnancy rate increased with the level of oocyte penetration reaching a maximum of 46% (17 of 35) in patients who exhibited an oocyte fusion rate of 75% to 100% or 44% (19 of 47) pregnant for those who exhibited a degree of polyspermy of more than one penetrating sperm per oocyte.

This relationship between the frequency distribution of the hamster oocyte penetration data and fertility was also reflected in significant differences between the pregnant and nonpregnant populations in terms of the overall distribution of the data (Mann-Whitney U test, $p = 0.0001$). The fertile population was characterized by a median (10th to 90th percentile) penetration rate of 78.9% (13.4% to 100%) with an average of 1.87 (0.13 to 4.88) spermatozoa penetrating each oocyte, and the nonpregnant group exhibited median values of 18.7% (0% to 93.7%) penetration and 0.2 (0 to 2.8) sperm per oocyte, respectively.

Life table analysis also confirmed the significant difference in cumulative pregnancy rates for men who exhibited different levels of hamster oocyte fusion. For the purpose of this analysis, an arbitrary threshold of 10% oocyte fusion was used to divide the patient population into two fractions according to the precedent set by Corson et al.[6] This analysis revealed significantly different ($p < 0.001$) monthly fecundity values of 0.0017 for the patients who exhibited less than 10% sperm oocyte fusion and 0.0081 for cases in which more than 11% of the oocytes were penetrated (Fig. 6).

Analysis of the conventional semen profiles for these

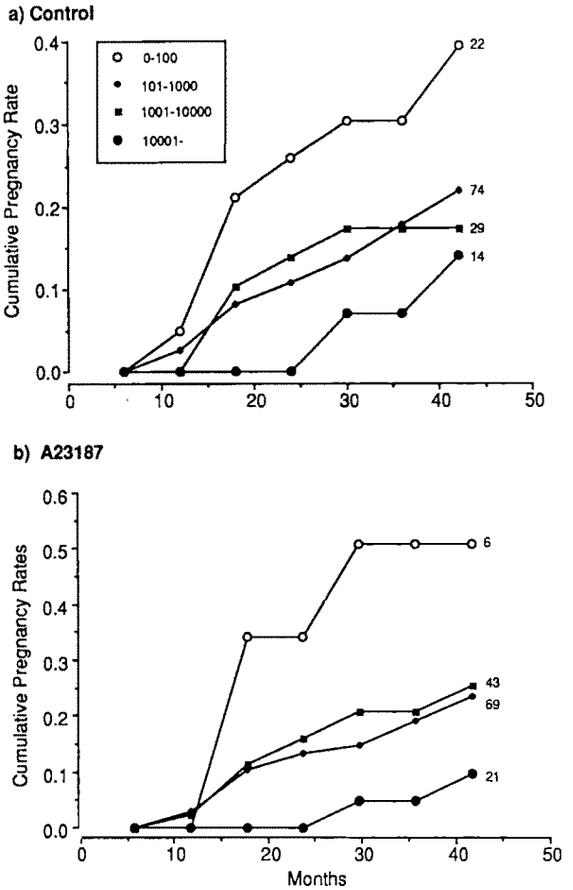


Fig. 4. Cumulative pregnancy rates for patients who exhibited different levels of reactive oxygen species generation in **(a)** absence and **(b)** presence of ionophore, A23187.

two groups of patients revealed significant differences ($p < 0.001$) in the central tendencies for all criteria (sperm concentration, total count, motility, morphology) that were assessed, although considerable overlap was observed at the extremes of the frequency distributions. Hence, although the median (10th to 90th percentile) values for sperm concentration, motility, and morphology for the patients who exhibited <10% hamster oocyte fusion were 33 (6 to 84) $\times$ 10^6 cells/ml, 47 (25% to 62%) and 39 (24% to 58%), respectively, the equivalent values for patients who exhibited more than 11% oocyte fusion were 69 (27 to 165) $\times$ 10^6 cells/ml, 55% (37% to 66%), and 56% (33% to 69%).

Conventional semen profile. There was no significant difference in the distribution of motility data between the pregnant and nonpregnant groups (Mann-Whitney U test), and the incidence of pregnancy was not significantly different (χ^2 analysis) between the patients who exhibited asthenospermia (<40% motility) (5 of 26; 19%) and the remainder of the study population (27 of 113; 24%).

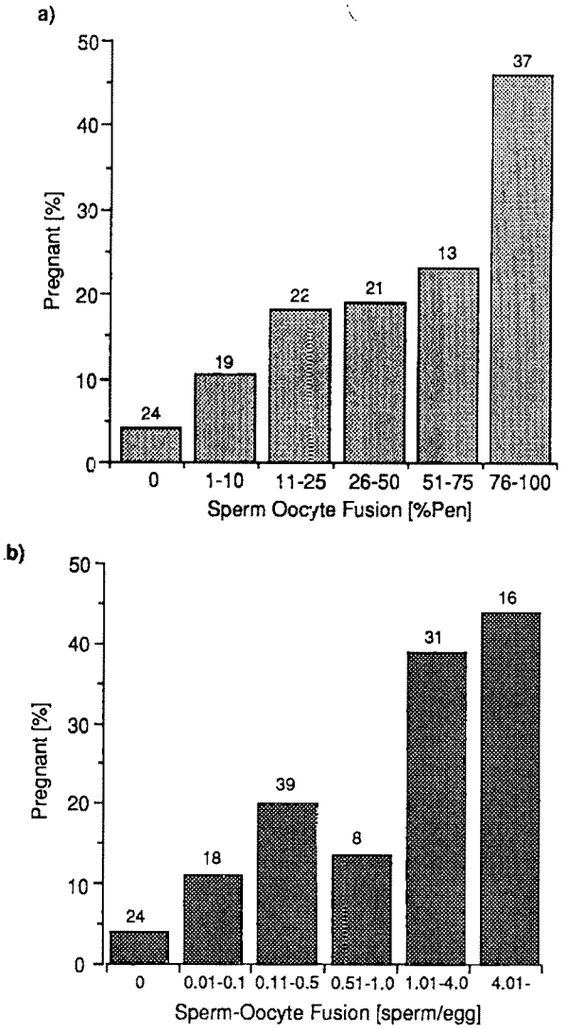


Fig. 5. Incidence of spontaneous pregnancy in relation to levels of sperm-oocyte fusion in hamster-oocyte penetration test expressed as **(a)** percentage of oocytes penetrated and **(b)** mean number of fusions per oocyte. *% Pen,* Percentage penetrated.

In contrast, there was a significant difference in the percentage of cells that exhibited a normal morphology in the pregnant and nonpregnant populations ($p = 0.01$; Mann-Whitney U test). However, if the conventional threshold of normality of (>40%) normal morphology was applied to these data, then the incidence of pregnancy was found to be no different (χ^2 analysis) in the teratospermic (<40% normal morphology) population (9 of 47; 19.1%) than in the remainder of the study population (20 of 69; 28.9%). Furthermore, life table analysis gave similar monthly fecundity values for patients who exhibited teratospermia (monthly fecundity = 0.0047) compared with the remaining population (monthly fecundity = 0.0071; Fig. 7, *a*).

Similar data were obtained for the relationship between sperm concentration and fertility. Hence, although the overall distribution of sperm concentration values in the pregnant and nonpregnant populations was significantly different (Mann Whitney U test; $p = 0.01$) the incidence of pregnancy in the patients who exhibited oligospermia (<20 × 10^6 cells/ml) (3 of 17; 17.6%) was not significantly different (χ^2 analysis) from the remainder of the study population (26 of 122; 21.3%). Furthermore, the monthly fecundity value for the patients who exhibited oligospermia (monthly fecundity = 0.0047) was not significantly different from that of the rest of the study population (monthly fecundity = 0.0063; Fig. 7, *b*).

Comment

This study has generated unique information on the prognostic value of conventional, biochemical, and functional techniques for the diagnosis of male fertility. The power of this analysis lies in the prospective design of the study in which confounding factors such as empirical treatments and variations in the fertility of the female partners have, as far as possible, been ruled out. We know of no other studies in which these criteria for the evaluation of diagnostic techniques in the male patient have been met, despite the fact that such prospective studies represent the most powerful (and perhaps the only truly valid) means of assessment of the clinical utility of such tests.

Apart from the normality of the female partner, the only other selection criterion that was used in this study was that the male partner should not be azoospermic. As a consequence of this selection policy, the study population presented with a wide range of seminal phenotypes, including patients who would have been classified as abnormal according to the conventional criteria of semen quality by virtue of deficiencies in the number, motility, or morphology of the spermatozoa. Life table analysis indicated that patients who were classified as abnormal under these circumstances were no less fertile than the remainder of the study population, which emphasizes, once again, the limited diagnostic value of the conventional semen profile.

In contrast, the zona-free hamster oocyte penetration test was shown to be of significant prognostic value in this prospective study. This particular test has been criticized repeatedly since its introduction because of a lack of standardization and poor quality control.[4] Standardization of the methodology is an issue that the World Health Organization has attempted to address by publishing a concensus protocol that was produced by consultation with experts in this particular field.[5] Despite the value of such a standardized protocol for the routine laboratory practice of the hamster oocyte penetration test, its biologic basis resides in the extremely low incidence of spontaneous acrosome reac-

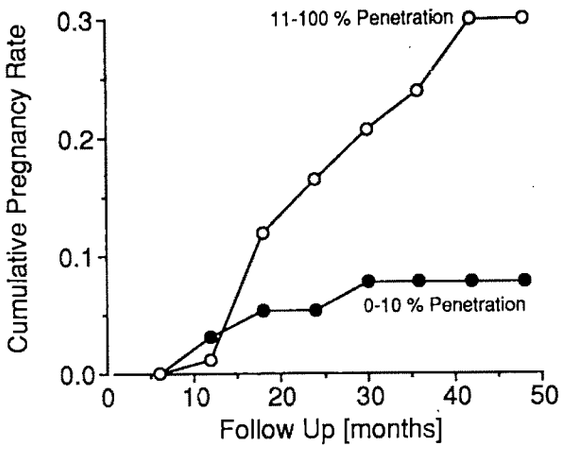


Fig. 6. Cumulative pregnancy rates for patients who exhibited sperm-oocyte fusion rates of more or less than 10%.

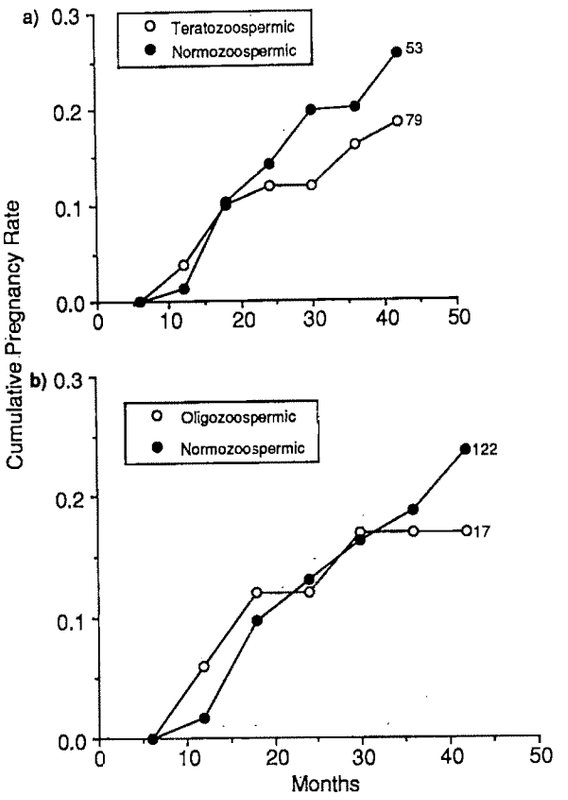


Fig. 7. Cumulative pregnancy rates for patients who exhibited **(a)** levels of normal sperm morphology of more or less than 40% and **(b)** sperm concentration of more or less than 20×10^6 cells/ml.

tions that occur in populations of human spermatozoa that are incubated for prolonged periods of time in vitro. We have therefore introduced a variation of this test procedure in which acrosome reactions are artificially induced by the ionophore A23187, which stimulates a simultaneous calcium influx into and a proton efflux from these cells. In response to this reagent, significantly enhanced levels of sperm-oocyte fusion are observed with sperm suspensions that have been obtained from normal fertile donors. In contrast, spermatozoa from male patients who exhibited overt evidence of infertility (e.g., oligospermia) produce low levels of sperm-oocyte fusion in the presence of A23187, which indicates that there are defects in these spermatozoa downstream from second messenger generation.[9]

The A23187-enhanced zona-free hamster oocyte penetration test should therefore be regarded as a variant of the basic assay that specifically generates information of the ability of human spermatozoa to respond to the calcium and pH signals that are generated by the ionophore with sperm-oocyte fusion. Because changes in intracellular calcium and pH seem to be the physiologic signals that activate mammalian spermatozoa in the presence of the zona pellucida, this version of the assay is not without a sound biologic foundation.[10, 18]

In previous studies we showed that the A23187-enhanced hamster oocyte penetration test gives an accurate indication of relative fertility in the settings of both artificial insemination by donor[19] and in vitro fertilization.[20] In the present investigation we confirmed the diagnostic value of this version of the test in a prospective study that focused on the incidence of spontaneous pregnancy. The results indicate the existence of a clear positive relationship between the levels of hamster-oocyte fusion and fertility with a low incidence (4%) of false-negative results, that is, patients who exhibit 0% sperm-oocyte fusion who achieve pregnancy.

This low incidence of false-negative results is extremely important because it is the 0% scores that, in principle, bear heavily on patient management. The validity of such scores depends critically on the quality of the assay and the nature of the protocol. In terms of quality, it is imperative that appropriate quality control measures be taken, including the inclusion of positive, fertile samples with every assay. In terms of protocol, the addition of A23187 appears to increase the sensitivity and predictability of the assay as judged from the low incidence of false-negative results and the improved segregation of patients who exhibited high and low fertility. Hence, although Corson et al. observed a twofold difference in the monthly fecundity rates between patients who exhibited penetration rates of more or less than 10% in an assay that lacked ionophore, we observed a fivefold difference in monthly fecundity rates between patients who exhibited such scores in the presence of A23187.

The major problem with the hamster oocyte test is

that it is a complex bioassay that only dedicated gamete biology laboratories can perform with precision. The principal role of this test should perhaps be as a bioassay of human sperm function in fundamental studies that are designed to elucidate the biochemical basis of defective sperm function. Once this objective has been achieved, the bioassay may be replaced by biochemical criteria that are more suited to routine diagnostic application.

Impaired fusion with zona-free hamster oocytes may involve multiple causes, and so it is doubtful whether the information that is generated by this complex bioassay could ever be substituted by a single chemical assessment. However, our recent studies identified at least one cause of poor sperm-oocyte fusion that might constitute the first of a battery of biochemical tests for the assessment of human sperm function. In these cases, the causative mechanism involves the induction of peroxidative damage to the unsaturated fatty acids that predominate in the sperm plasma membrane as a result of exposure to high levels of hydrogen peroxide and oxygen free radicals.[11-13, 21] In this study we examined the oxidative stress to which spermatozoa are subjected with the use of a simple chemiluminescent assay for reactive oxygen species, which is based on the oxidation of luminol.[11] The results generated with this technique exhibited a negative correlation with sperm-oocyte fusion rates, as observed in previous studies.[11-13] Furthermore, a negative correlation was also observed between the levels of reactive oxygen species generation and the incidence of spontaneous pregnancy in vivo. The chemiluminescent signals that were generated by the washed sperm preparations were particularly informative at the extreme ends of the frequency distribution. Samples that exhibited basal chemiluminescent signals of <100 counts per 10 seconds were three to four times more likely to achieve a pregnancy than those that exhibited signals >10,000 counts per 10 seconds. If the chemiluminescent signal remained <100 counts per 10 seconds after addition of A23187, the patients' monthly fecundity value was about seven times higher (monthly fecundity values of 0.016 compared with 0.0023) than that of those patients who exhibited counts >10,000 per 10 seconds after ionophore treatment. The measurement of reactive oxygen species generation by the washed ejaculates therefore provides data of prognostic value, albeit for the subsets of patients who exhibited extremely high or low levels of activity.

Nevertheless, it must be emphasized that the observed relationship between reactive oxygen species generation and cumulative pregnancy rates was achieved in a cohort of patients for which the conventional semen profile per se was of no prognostic value. Furthermore, the patient population contained only 21 patients in whom high levels of reactive oxygen species generation were associated with low fertility (Table II). If only patients who exhibited oligospermia had been selected, then the proportion of samples that exhibited high levels of reactive oxygen species generation would have been considerably higher.[13] In addition, it must be remembered that lipid peroxidation is also dependent on the capacity of the spermatozoa or seminal plasma to scavenge reactive oxygen species as well as on the degree of exposure to free radical attack. Simultaneous measurement of defensive enzyme systems such as superoxide dismutase may therefore shed further light on the oxidative stress that is experienced by the spermatozoa, as might the direct measurement of lipid peroxides or conjugated dienes.

In summary, this article describes a unique data set in which the conditions for a valid assessment of new andrologic tests have been met in a prospective paradigm, which focused on the incidence of spontaneous pregnancy in a cohort of untreated couples in which a female factor has, as far as possible, been excluded. The results that were obtained indicate that sperm function tests, including assessments of sperm-oocyte fusion and reactive oxygen species generation, constitute a significant improvement over the conventional semen profile in the accurate assessment of male fertility.

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The hemodynamic effects of intubation during nitroglycerin infusion in severe preeclampsia

Stephen Longmire, MD,[a] Line Leduc, MD,[b] Monica M. Jones, MD,[a] Joy L. Hawkins, MD,[a] Thomas H. Joyce III, MD,[a] and David B. Cotton, MD[b]

Houston, Texas

The effectiveness of intravenous nitroglycerin infusion in lowering maternal blood pressure and in blunting the hemodynamic responses to endotracheal intubation was evaluated in six primigravid women with severe preeclampsia. Monitoring consisted of continuous electrocardiogram monitoring, arterial cannulation, and flow-directed pulmonary arterial catheterization in each patient. All patients underwent oxytocin induction of labor and crystalloid and/or colloid expansion to produce a pulmonary capillary wedge pressure of 10 to 15 mm Hg and a colloid osmotic pressure of >17 mm Hg. Intravenous nitroglycerin was administered before induction of general anesthesia. The hemodynamic effects associated with endotracheal intubation revealed a change in the heart rate from 104 ± 10 to 133 ± 17 beats/min, an increase in mean arterial pressure from 134 ± 12 to 164 ± 32 mm Hg, and an increase in systemic vascular resistance from 1262 ± 342 to 1351 ± 259 dynes-sec-cm^{-5} that was accompanied by a small change in the cardiac index from 4.5 ± 1.2 to 4.5 ± 0.9 L · min^{-1} · m^{-2}. (Am J Obstet Gynecol 1991;164:551-6.)

Key words: Obstetric anesthesia, pregnancy-induced hypertension, intubation complications

The risks associated with the pressor response to endotracheal intubation in the general surgical patient have long been appreciated.[1-9] However, only recently has it been realized that this response may result in acute left ventricular failure, pulmonary edema, cerebral hemorrhage, and eclamptic seizures in the patient with severe pregnancy-induced hypertension.[4, 9-13]

Intravenous nitroglycerin has been recommended as an antihypertensive agent for use during endotracheal intubation in the treatment of severe preeclampsia.[14-16] Nitroglycerin would be expected to work well in a volume-contracted population such as that seen in this disorder. However, profound hypotension can be seen with the use of nitroglycerin in hypovolemic patients. To prevent abrupt and profound hypotensive re-

From the Departments of Anesthesiology[a] and Obstetrics and Gynecology,[b] Baylor College of Medicine.
Received for publication January 9, 1990; accepted August 31, 1990.
Reprint requests: David B. Cotton, MD, Department of Obstetrics and Gynecology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.
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sponses that adversely affect uteroplacental blood flow during vasodilator therapy, we often volume-expand patients with severe preeclampsia. Volume expansion, however, may result in attenuation of the hypotensive action of a drug such as nitroglycerin, which is primarily a venodilator. We undertook this study to document the hemodynamic efficacy of intravenous nitroglycerin when used to control the circulatory responses to endotracheal intubation in volume-expanded patients with severe preeclampsia.

Material and methods

The Baylor College of Medicine Institutional Review Board for Human Investigation approved this study, and all patients gave written informed consent. Enrolled in this study and admitted to the obstetric intensive care unit were six patients with severe preeclampsia with a blood pressure of at least 160/110 mm Hg, accompanied by hyperreflexia and proteinuria (2+). The maternal age ranged from 17 to 20 years, and gestational age ranged from 32 to 41.5 weeks. None of these patients had received prenatal care. Left uterine displacement of at least 15 degrees was maintained throughout the study. These patients did not receive any medication other than magnesium sulfate (4 to 6 gm intravenous loading dose, 2 to 3 gm/hr maintenance infusion) and oxytocin before enrollment in the study. Oxytocin augmentation of labor was initiated when volume expansion was achieved.

Maternal systemic and pulmonary arterial pressures, central venous pressure, electrocardiogram, cardiac output, and uterine contractions, as well as fetal heart rate, were recorded on a Hewlett-Packard eight-channel physiologic recorder. Pressures were transduced with the Hewlett-Packard model 1290A quartz transducers, which were zeroed at the level of the right atrium. Radiographic verification of the position of the pulmonary artery catheter tip was established. Each pressure was measured with a Hewlett-Packard model 78231C cardiac output computer. Five determinations were made, each of which used 5 ml injections of 5% dextrose solution at 0° C. The highest and lowest values obtained were discarded, and the remaining three were averaged. To eliminate hypovolemia and low plasma colloid osmotic pressure as confounding variables and with the hope of preserving uteroplacental blood flow, patients received crystalloid such as lactated Ringer's solution and/or colloid (5% or 25% albumin). The goal of volume expansion was to achieve a pulmonary capillary wedge pressure of 10 to 15 mm Hg and a colloid osmotic pressure >17 mm Hg before endotracheal intubation during nitroglycerin infusions. Intravenous nitroglycerin was mixed in glass bottles of 5% dextrose in water and was infused via Travenol 2C9096 tubing with a calibrated infusion pump.

Baseline measurements were determined after arrival of the patient in the operating room. Infusion of intravenous nitroglycerin was begun at 0.1 μg/kg/min. The nitroglycerin infusion was titrated at 3-minute intervals until either a 20% reduction of the mean arterial pressure had been achieved or signs of fetal or maternal distress occurred.

Patients were then preoxygenated for a period of 10 minutes, during which time the infusion rate of the intravenous nitroglycerin was further titrated in an attempt to produce a further reduction of 20% in the mean arterial pressure. (Generally, this reduction could not be achieved because of the development of maternal nausea and vomiting.)

Induction measurements were made immediately before rapid-sequence intravenous induction-intubation, which was accomplished with thiopental 3 mg/kg and succinylcholine 100 mg. Maintenance anesthesia was provided with oxygen/nitrous oxide (4:4 L/min), halothane 0.5%, and succinylcholine 0.2% infusion. Laryngoscopy-to-intubation time did not exceed 33 seconds in any patient, and Miller 2 blades and 7.0 mm inner diameter cuffed endotracheal tubes were used throughout the study. The average duration between intubation and delivery was 4 minutes 38 seconds.

Intubation hemodynamics were measured as the endotracheal tube was inserted. The pulmonary artery catheter's balloon was briefly inflated, then deflated, and a single determination of the cardiac output was made. When the umbilical cord was clamped, morphine 200 μg/kg was given intravenously, the oxygen flow was reduced to 2 L/min, and the halothane was gradually discontinued.

Delivery measurements were made immediately after manual extraction of the placenta. A final set of measurements was made 30 minutes after surgical closure.

Intravenous nitroglycerin was considered to have been effective if the increase in blood pressure and/or heart rate did not exceed 20% of the baseline.

Data were analyzed with the one-way analysis of variance procedure in the SPSS/PC software package (SPSS Inc, Chicago). Multiple comparisons were tested for significance with Duncan's procedure.[17] In all cases significance was accepted at the $p < 0.05$ level. All data are reported as mean ± SD unless otherwise specified.[18]

Results

Mean maternal height and weight were 164.1 ± 3.8 cm and 82.4 ± 15.3 kg, respectively. The mean body surface area was 1.9 ± 0.19 m^2. The maternal hemodynamic effects observed at each interval are presented in Table I. Initial examination of the table appears to indicate that the original protocol was not followed. Indeed, the 20% reduction in mean arterial pressure

Table I. Maternal hemodynamic effects of intravenous nitroglycerin

	Operating room baseline	*Induction*	*Intubation*	*Delivery*	*30 min after delivery*
BSA (m^2)	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2
HR (beats/min)	104 ± 10	114 ± 20	133 ± 17*	111 ± 15	89 ± 7*
MAP (mm Hg)	134 ± 12	134 ± 10	164 ± 32*	134 ± 16	132 ± 9
CVP (mm Hg)	7 ± 5	6 ± 4	8 ± 5	9 ± 6	4 ± 3
PAM (mm Hg)	18 ± 8	19 ± 8	24 ± 12	24 ± 8*	15 ± 4
PCWP (mm Hg)	14 ± 7	13 ± 7	15 ± 6	19 ± 8	10 ± 3
CI ($L/min/m^2$)	4.5 ± 1.2	4.5 ± 0.9	4.5 ± 0.9	5.2 ± 0.3	4.0 ± 0.7*
SVR (dynes · sec · cm^{-5})	1262 ± 342	1240 ± 278	1351 ± 259	932 ± 228	1373 ± 231

All values are mean ± SD. *BSA,* Body surface area; *HR,* heart rate; *MAP,* mean arterial pressure; *CVP,* central venous pressure; *PAM,* pulmonary artery mean pressure; *PCWP,* pulmonary capillary wedge pressure; *CI,* cardiac index; *SVR,* systemic vascular resistance.

*Significant ($p < 0.05$) compared with baseline value.

Table II. Neonatal data

Infant No.	*Gestational age (wk)*	*Birth weight (gm)*	*Apgar score*		*Umbilical artery pH*	*Umbilical vein pH*
			1 min	*5 min*		
1	34	2300	5	8	7.30	7.35
2	41	3590	8	9	7.23	7.31
3	37	2970	8	9	7.25	7.34
4	38	2480	8	8	7.27	7.28
5	34	1540	7	8	7.32	7.33
6	36	2160	7	8	7.30	7.32

we hoped for was not present before intubation (no interval is significantly less than baseline). Close inspection of the protocol, however, reveals that our titration was limited by the development of either maternal nausea and vomiting or by fetal bradycardia in one case. Neonatal data are presented in Table II. All six patients exhibited progressively flattened beat-to-beat variability of the fetal heart rate recording. Whether magesium sulfate or nitroglycerin was responsible for the decrease in beat-to-beat variability remains unclear. In one patient fetal bradycardia necessitated stopping the infusion. In spite of the continuous presence of poor beat-to-beat variability, only neonate No. 1 had a 1-minute Apgar score <7. Neonate No. 5 had the highest umbilical venous methemoglobin concentration (4.6%) in the study. This neonate's mother had received one of the highest doses of intravenous nitroglycerin that was administered during the study (up to 30 μg/kg/min).

During initial titration, in four of six patients studied, nausea and vomiting developed and necessitated a reduction in the dose of intravenous nitroglycerin. None of these patients had experienced a 20% reduction in the mean arterial pressure before nausea and vomiting developed. Only one patient complained of a headache at the same time that nausea and vomiting occurred. Attempts to acutely reduce maternal mean arterial pressure in the operating room before induction of anesthesia were also characterized by maternal nausea, retching, and vomiting. The onset of nausea and vomiting did not appear to be related to the dose of nitroglycerin. In only two of six patients were we able to prevent an increase in mean arterial pressure >20% of baseline. Because of the high incidence of nausea and vomiting, coupled with an efficacy of only 33% in preventing an increase in mean arterial pressure that was >20% baseline, we terminated the protocol after only six of the projected 10 patients had been studied. All patients were delivered of infants by cesarean section. The indications for cesarean section were fetal bradycardia, (one case); cephalopelvic disproportion, (three cases); failure of induction, (one case); and worsening signs of HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), (one case).

Comment

In women with severe preeclampsia, induction of general anesthesia and endotracheal intubation significantly increase blood pressure, pulmonary arterial pressure, and pulmonary capillary wedge pressure. This response may precipitate any of several untoward events, including cardiac failure and pulmonary edema, cerebral edema, and/or hemorrhage.[9-13, 19] The reflex increase in the maternal heart rate and blood pressure that results from endotracheal intubation may play a role in the genesis of these complications. Indeed, a correlation between changes in mean arterial pressure that occurs during laryngoscopy and in plasma cate-

Table III. Amount of volume expansion and dose of nitroglycerin

Patient No.	*Blood pressure before volume expansion*	*Volume (ml)*	*Blood pressure after volume expansion*	*Blood pressure with nitroglycerin*	*Nitroglycerin dose (μg/kg/min)*
1	197/104	1500 ml 5% albumin	206/95	204/87	2.02
2	184/96	100 ml 25% albumin 500 ml crystalloid	194/96	184/88	15.38
3	200/112	250 ml 5% albumin	208/116	168/96	5.00
4	168/104	1250 ml 5% albumin	200/104	160/92	35.29
5	224/124	1500 ml 5% albumin 50 ml 25% albumin	192/100	192/104	33.33
6	192/88	350 ml crystalloid	208/96	192/112	0.44

cholamine concentration has been previously observed.[20]

Several investigators have utilized multiple agents to decrease the blood pressure response associated with intubation. An ideal drug should be potent, rapidly acting, controllable, and without detrimental maternal or fetal side effects.

Hodginkson et al.[13] have previously shown that hydralazine (the most commonly used antihypertensive agent in severe preeclampsia) does not adequately control the brief but sudden episode of hypertension that occurs during intubation. The onset of action of hydralazine is too slow, and repetitive administration of hydralazine in an effort to control blood pressure may result in excess maternal hypotension and fetal distress.[21, 22]

Nitroprusside has also been used to control the hemodynamic changes associated with intubation. The potent and rapid onset of action, combined with no adverse effects on uterine blood flow,[15] support the use of this agent. However, nitroprusside can cause fetal toxicity in excessively large doses[23, 24] and can easily cause hypotension if not given carefully. Other potential maternal side effects include significant reflex tachycardia and the inhibition of cerebral autoregulation.[25] This latter effect predisposes a patient to elevations in intracranial pressure in cases of breakthrough hypertension. The appropriate role of nitroprusside in severe preeclampsia complicated by cerebral edema is still unclear.

Trimethaphan,[26] a ganglionic blocking drug, has limited use because of its slow onset and long duration of action. Furthermore, its inhibition of the action of pseudocholinesterase can prolong paralysis after succinylcholine administration. Also the mydriasis seen after trimethaphan administration makes neurologic assessment in complicated cases difficult.[26]

Labetalol,[27] a selective α_1-adrenergic receptor and a nonselective β-adrenergic receptor–blocking agent with intrinsic β_2-agonist activity, has been used in the treatment of patients with preeclampsia.[22, 27] Ramanathan et al.[27] reported that labetalol administration effected a moderate reduction in mean arterial pressure and tachycardic response to intubation in patients with mild to moderate preeclampsia. However, these authors were unable to achieve a 20% reduction in mean arterial pressure with labetalol before induction of anesthesia in 80% of patients because of a large variation in dose requirements among patients. They concluded that higher doses of labetalol might be needed in many patients. To date, no significant deleterious fetal effects have been reported to result from labetalol administration.

Nitroglycerin has been reported effective in controlling blood pressure in severe preeclamptic patients who undergo general anesthesia for cesarean section.[14-16] Our results contradict those obtained by Hood et al.,[16] who concluded that intravenous nitroglycerin was effective in controlling perioperative blood pressure. However, Hood et al.[16] were able to achieve nearly a 20% reduction of mean arterial pressure before induction of anesthesia, whereas we only achieved a mean reduction of 13%. In the study by Hood et al.,[16] other differences were that Hood's patients had received prenatal care, were significantly less hypertensive, and had not received volume loading before the administration of nitroglycerin. In addition, their patients received hydralazine during labor.

We defined a therapeutic failure as any increase in heart rate or blood pressure of >20% over baseline. With this definition, maximal therapy with intravenous nitroglycerin failed to control the heart rate in all patients in our study. Intravenous nitroglycerin failed to control blood pressure in 66% of the patients, and the

dose used was of no value in predicting the ability to control blood pressure during intubation. A possible explanation for our failure to achieve the desired 20% reduction in mean arterial pressure was that our patients were all volume-expanded (Table III), as opposed to those of Hood et al.[16] In our study we volume-expanded patients to maintain an adequate maternal cardiac output and therefore to preserve the uteroplacental blood flow while reducing blood pressure. This approach also eliminated hypovolemia and low colloid osmotic pressure as confounding variables in an already complex investigation.

In agreement with our previous published data, it seems that volume expansion blunts the action of nitroglycerin, since the agent primarily affects the venous capacitance and ventricular preload.[28] We did, however, prevent a 20% increase in mean arterial pressure at the time of intubation in 33% of the patients. Nevertheless, the mean value for the study group of 164 ± 32 mm Hg was substantially more than the 140 mm Hg level that has been associated with loss of cerebrovascular autoregulation.[25] This raises a concern regarding the use of intravenous nitroglycerin to treat severe preeclampsia complicated by cerebral edema.

The most common side effects of intravenous nitroglycerin treatment reported by our patients were nausea and vomiting. The diluent used in preparation of intravenous nitroglycerin contains 70% ethanol,[29] which may have contributed to the development of these symptoms. Furthermore, an increase in intracranial pressure related to nitroglycerin might explain these side effects, although only one patient complained of headache during nitroglycerin infusion.

Our patients were monitored in the obstetric intensive care unit, where the infusion of intravenous nitroglycerin was initiated before surgery. Fetal heart rate monitoring was used in every case. The flattening of the beat-to-beat variability reported in our patients could be related to nitroglycerin infusion. It is unlikely, however, that these fetal heart rate changes indicated chronic fetal distress, since none of the umbilical cord gas levels showed a pH <7.25 and only neonate No. 1 had a 1-minute Apgar score <7.

It is of interest that central venous and pulmonary artery pressures increase with delivery of the placenta, while blood pressure decreases transiently. We speculate that the increase in venous return to the heart is secondary to a shift of blood from the uterus to the intravascular space, the release of caval compression from the gravid uterus, and mobilization of extravascular fluid into the intravascular compartment. This probably accounts for the increase in the central venous pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure seen at the time of delivery.

In summary, we found that intravenous nitroglycerin was not effective in controlling the hypertensive response to endotracheal intubation in our population of volume-expanded patients with severe preeclampsia. Given the highly variable responses to intravenous nitroglycerin as an antihypertensive agent, we conclude that the routine use of intravenous nitroglycerin to combat the hypertensive response to endotracheal intubation in patients with severe preeclampsia may not be warranted if the patient has been previously volume-expanded. In patients who are volume-contracted with severe preeclampsia, nitroglycerin may still be a useful agent for the control of hypertension.

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Vasoactive intestinal polypeptide loses its ability to increase vaginal blood flow after menopause

Connie Palle, MD,[a] Helle E. Bredkjær, MD,[a] Jan Fahrenkrug, PhD,[a] and Bent Ottesen, PhD[b, c]

Copenhagen, Denmark

The effect of vasoactive intestinal polypeptide on vaginal blood flow was investigated in postmenopausal women. In women who were receiving hormonal replacement therapy the vasodilatory response induced by vasoactive intestinal polypeptide was identical to that of young women, whereas in postmenopausal women who were receiving no replacement therapy, the response induced by vasoactive intestinal polypeptide was absent. Plasma levels of vasoactive intestinal polypeptide and systemic cardiovascular effects were identical in the two groups. (AM J OBSTET GYNECOL 1991;164:556-8.)

Key words: Vasoactive intestinal polypeptide, vaginal blood flow, postmenopausal women, hormonal replacement therapy

In the female genital tract vasoactive intestinal polypeptide is regarded as a neurotransmitter involved in the control of blood flow, motility, and secretion.[1] Exogenous vasoactive intestinal polypeptide has been shown to increase vaginal blood flow and lubrication in fertile, nonpregnant women to a level similar to that observed during sexual arousal.[2] In the present study, we investigated the vasodilatory effect of vasoactive intestinal polypeptide in two groups of postmenopausal women. The first group included six women (age [mean ± SEM], 58 ± 1 years; body weight, 66.0 ± 5.7 kg; duration of menopause, 7.7 ± 2.6 years) who were receiving no medication at all. The second group consisted of six postmenopausal women (age, 54 ± 1.2 years; body weight, 61.0 ± 4.4 kg) who were receiving hormonal replacement therapy for climacteric symptoms. Five of the women had therapy including both estrogen and gestagen, whereas one woman who had had a hysterectomy received only estrogen. The average time of treatment was 4.3 ± 1.4 years. All women gave informed consent after the study was approved by the local ethical committee of Copenhagen, Denmark.

From the Departments of Clinical Chemistry[a] and Gynaecology,[b] Bispebjerg Hospital, and the Department of Gynaecology and Obstetrics,[c] Copenhagen Hospital in Herlev.
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Reprint requests: Connie Palle, MD, Department of Clinical Chemistry, Bispebjerg Hospital, DK-2400 Copenhagen NV, Denmark.
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Table I. The systemic cardiovascular effects of vasoactive intestinal polypeptide were observed to the same extent in the two different groups of postmenopausal women

	Blood pressure (mm Hg)		*Pulse frequency (per min)*
	Systolic	*Diastolic*	
Before VIP			
No treatment	123 ± 4	78 ± 4	73 ± 3
Hormone treatment	116 ± 5	78 ± 2	70 ± 4
VIP (10 μg)			
No treatment	115 ± 5	63 ± 4*	89 ± 6*
Hormone treatment	109 ± 6	70 ± 2*	90 ± 5*

Data represent mean ± SEM (n = 6).

VIP, Vasoactive intestinal polypeptide.

*Indicates a significant difference from before VIP, $p < 0.05$.

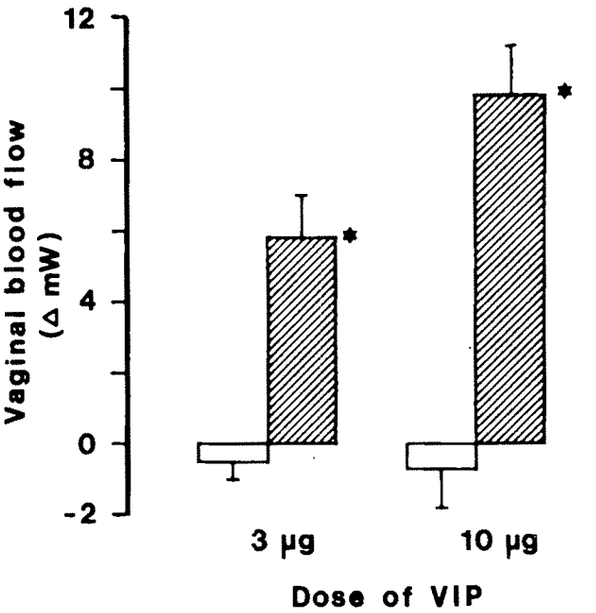


Fig. 1. Effect of vasoactive intestinal polypeptide on vaginal blood flow in postmenopausal women receiving no medication *(open bars)* and in postmenopausal women receiving hormonal replacement therapy *(hatched bars)*. Figures are given as means ± SEM (n = 6). Infusion of saline solution did not change vaginal blood flow (not shown). *VIP*, Vasoactive intestinal polypeptide. *Asterisks* indicate significant difference from before injection of vasoactive intestinal polypeptide, $p < 0.05$.

Local vaginal blood flow was continuously measured by a heated oxygen electrode (TMC 2, Radiometer, Copenhagen, Denmark),[3] that was placed on the right side of the vaginal wall, approximately 5 cm from the introitus. The electrode was kept in place by a slight vacuum. The vaginal blood flow was measured by the energy (in milliwatts) needed to maintain the electrode at a constant temperature (42° C). The change in energy is proportional to the change in vaginal blood flow. The blood flow changes are given as the increase or decrease in milliwatts and were recorded on a TMC 2 pen recorder (Radiometer). The basal vaginal blood flow was measured for 30 minutes, whereupon synthetic human vasoactive intestinal polypeptide (Peninsula Laboratories, St. Helens, United Kingdom) dissolved in isotonic saline solution and then sterile filtered was administered as intravenous injections in doses of 0, 3, and 10 μg in one arm. From the other arm, blood samples were collected (1, 2, 3, 4, 5, and 10 minutes after the injections) for analysis of vasoactive intestinal polypeptide by radioimmunoassay. Blood pressure and pulse frequency were monitored throughout the experiment.

In the untreated group of women vasoactive intestinal polypeptide was unable to increase vaginal blood flow, whereas in the hormone-treated group of postmenopausal women a significant ($p < 0.05$; Mann-Whitney u test) increase in vaginal blood flow was observed after intravenous injection of both 3 and 10 μg of vasoactive intestinal polypeptide (Fig. 1). No difference was observed in basal vaginal blood flow between the two groups (untreated group, 135 ± 3 mW; treated group, 133 ± 4 mW). The two groups showed identical increments in plasma vasoactive intestinal polypeptide concentrations. After intravenous injection of 10 μg of vasoactive intestinal polypeptide, the peak concentrations in the untreated and the hormone-treated group were 151 ± 32 pmol/L and 96 ± 18 pmol/L, respectively, values that were not significantly different. Basal concentrations were <20 pmol/L in all women. Systemic cardiovascular side effects (i.e., diastolic hypotension, tachycardia, and cutaneous flushing localized to the face and upper trunk) were observed to the same extent in the two groups of women (Table I).

The present study indicates that vasoactive intestinal polypeptide loses its ability to increase vaginal blood flow after menopause. However, when women were treated with sex hormones, the vasodilatory effect seemed to be maintained or returned to a level that corresponded to that of fertile women. It has been suggested that vasoactive intestinal polypeptide is involved in the physiologic vasodilation and vaginal lubrication that take place during sexual arousal. Vaginal dryness is a common problem in postmenopausal women, and it is well known that estrogen improves this symptom. The function of the vascular vasoactive intestinal polypeptide receptor in the vagina seems to depend on the presence of sex hormones, and the mechanism may be that sex hormones accelerate the synthesis of the vasoactive intestinal polypeptide receptor. However, the systemic cardiovascular effects of vasoactive intestinal polypeptide were unaffected in postmenopausal women. In agreement with our results, it was recently discovered that vasoactive intestinal polypeptide loses its prolactin-releasing activity in postmenopausal

women.[4] The interaction among vasoactive intestinal polypeptide, its receptor, and sex hormones in the female reproductive system must be further elucidated.

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Analysis of antigen expression at multiple tumor sites in epithelial ovarian cancer

Stephen C. Rubin, MD, Connie L. Finstad, PhD, William J. Hoskins, MD, Diane Provencher, MD, Mark G. Federici, BS, Kenneth O. Lloyd, PhD, and John L. Lewis, Jr., MD

New York, New York

The question of whether the antigenic phenotype of human epithelial ovarian cancer varies in a given patient between the primary tumor and metastatic sites or among metastatic sites themselves is an important issue in planning potential therapeutic strategies for ovarian cancer. We have obtained tumor specimens from at least two separate sites during operations on 12 patients with epithelial ovarian cancer, and we have typed these specimens with a group of 18 monoclonal antibodies that react with cell-surface glycoprotein and carbohydrate antigens, including blood group antigens. Antibodies with relative specificity for malignant cells as well as those that detect more widely distributed epithelial antigens were used. A total of 31 specimens from 12 patients with advanced adenocarcinoma (8 serous, 3 undifferentiated, 1 endometrioid) of the ovary were studied, including fresh ascites cells in two patients. Frozen sections of tumor specimens were stained with the antibodies by the indirect immunoperoxidase technique and graded semiquantitatively. Little difference was seen in antigenic expression of tumors that were obtained from various sites in the same patient for either the epithelial cell markers or blood group markers. Intratumoral antigenic heterogeneity was seen, but this was generally quite consistent within a given patient's specimens. As anticipated, variations in antigen expression were seen among specimens from different patients. The antigenic phenotype of the tumor specimens in a given patient, as determined immunohistochemically by our group of antibodies, showed only minor variation among primary and metastatic sites. (Am J Obstet Gynecol 1991;164:558-63.)

Key words: Ovarian cancer, immunology, antigen expression, antigenic heterogeneity

The development of monoclonal antibody technology has led to accelerated activity in the field of tumor immunology and increased the possibility that antibodies may one day play an important role in the management of cancer. In the field of gynecologic oncology a major portion of this activity has related to ovarian cancer for which there is a pressing need for improvements in early diagnosis, monitoring of response, and treatment. Many antibodies that react with ovarian cancers have been identified,[1] serum markers have come into clinical use,[2, 3] and preliminary studies of tumor imaging and therapy with monoclonal antibodies have been reported.[4] In spite of these efforts, many questions that relate to the clinical usefulness of antibodies remain unanswered. Among the major questions is the extent to which antigen expression varies between the primary tumor and metastatic sites and among meta-

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Reprint requests: Stephen C. Rubin, MD, 1275 York Ave., New York, NY 10021.
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Table I. Monoclonal antibodies

*Antibody**	*Immunoglobulin subclass*	*Antigen characterization*			
		Heat-stable†	*Periodate-sensitive‡*	*Neuraminidase-sensitive§*	*Antigen detected*
Antibodies detecting epithelial cell antigens					
MH99	IgG2a	−	−	−	gp (38kD/32+6kD, ESA)
T16	IgG2b	−	−	−	gp (48kD/42+6kD)
OC125	IgG1	+	−	−	gp (CA 125)
MW162	IgM	+	−	−	Mucin
E29	IgG2a	+	−	−	Mucin (EMA)
MX35	IgG1	+	−	−	Carbohydrate (?)
MW207	IgG1	−	−	−	gp (37kD)
MT334	IgG1	+	+	−	Mucin
B72.3	IgG1	+	+	+	Mucin (sialyl-Tn, TAG-72)
Antibodies detecting blood group–related antigens					
T174	IgG1	+	+	−	Le^a
19.9	IgG1	+	+	+	sialyl-Le^a
T218	IgM	+	+	−	Le^b
P12	IgM	+	+	−	Le^x
118	IgG3	+	+	−	Le^y
F3	IgM	+	+	−	Le^y+H type 2
RAI-15	IgM	+	+	−	H type 2
T36	IgG3	+	+	−	A
S8	IgM	+	+	−	B

gp, Glycoprotein.
*Original references can be found in Rubin et al.[5]
†Indicates carbohydrate or mucin epitope.
‡Indicates carbohydrate epitope.
§Indicates presence of sialic acid in epitope.

static sites themselves. This issue, which has significant implications regarding the selection of antibodies for clinical use, is of particular importance in ovarian cancer because most patients with ovarian cancer present with tumor that has spread to multiple metastatic sites. If a single sample of a patient's tumor is characterized antigenically, can it be assumed that tumor in other locations will show similar antigenic expression? To address this question we have obtained tumor specimens from several sites in a group of patients with ovarian cancer and studied these specimens with a panel of monoclonal antibodies that represent a spectrum of cell-surface glycoprotein–related, carbohydrate-related, and blood group–related antigens, some with relative specificity for malignant cells and others with a wider distribution of reactivity.

Patients and methods

Antibodies. Monoclonal antibodies MH99, T16, MW162, MW207, MX35, MT334, T174, T218, P12, 118, F3, T36, and S8 were generated and characterized in our laboratory. They are described in Table I and referenced fully in previous publications.[5] The monoclonal antibody RAI-15 was a gift from Dr. R. Ueda of Nagoya, Japan; OC125 was obtained from Dr. R. Bast of Duke University; B72.3 was obtained from Dr. J. Schlom of the National Cancer Institute; 19.9 was purchased from Centocor (Malvern, Pa.); and E29, which detects epithelial membrane antigen, was purchased from Dako Corp. (Santa Barbara, Calif.).

Immunohistochemical analysis. Tumor specimens were obtained at the time of laparotomy in 12 patients with epithelial cancer of the ovary. Specimens were obtained from three different tumor sites in five women and from two different sites in the remaining seven women. All metastatic tumor was obtained from sites within the peritoneal cavity. Additionally, tumor cells were obtained from ascitic fluid in two women after lysis of erythrocytes and centrifugation of the remaining cells. Tissue specimens and ascites cell pellets were frozen in liquid nitrogen, embedded in OCT compound, and stored at −70° C until they were sectioned. Frozen sectons were cut at 6 μm, fixed with acetone and paraformaldehyde, and treated with 1% hydrogen peroxide to eliminate endogenous peroxidase activity. Sections were stained with preparations of monoclonal antibodies (concentration of 20 μg/ml) by the indirect immunoperoxidase technique with diaminobenzidine as the chromogen and hematoxylin as a counterstain. Detailed methods have been described previously.[5] Sections were examined with a light microscope, and the antibody staining patterns for the approximately 700 sections were scored in a semiquantitative fashion by one person.

To test for heat stability of the antigens, antibodies were incubated for 1 hour at 4° C with a pellet of a

Table II. Clinical characteristics and tumor sites

Patient	*Age*	*Grade*	*Cell type*	*Primary sites**	*Metastatic sites*	*Ascites pellet*	*Prior chemotherapy*
N.A.	71	3	Pap. serous	1	1	1	None
L.C.	56	3	Undiff.	1	1	0	None
A.C.	45	3	Undiff.	1	1	0	None
S.G.	45	2	Undiff.	0	2	0	ADR, CTX,CDDP
M.J.	71	3	Pap. serous	1	1	0	None
B.K.	54	3	Pap. serous	2	1	0	None
R.L.	61	3	Serous	2	1	0	None
K.M.	35	1	Endometrioid	2	1	0	None
K.Mc.	46	3	Pap. serous	1	2	0	CTX,CDDP
C.M.	39	3	Pap. serous	2	0	1	None
A.O.	75	2	Pap. serous	0	2	0	ADR, CTX,CDDP
J.S.	72	3	Pap. serous	2	1	0	None

Pap. serous, Papillary serous; *Undiff.*, undifferentiated; *ADR*, doxorubicin; *CTX*, cyclophosphamide; *CDDP*, cisplatin.
*Each ovary considered as separate site.

human cancer cell line that showed positive antibody reactivity or with the cell pellet after it had been boiled (100° C) for 5 minutes. The two samples of absorbed monoclonal antibodies were tested for reactivity by immunohistochemistry and were compared with the reactivity of the same unabsorbed monoclonal antibody. Monoclonal antibodies that detected heat-stable antigens showed no reactivity on tissue sections after absorption with a boiled cell pellet.

To test for carbohydrate epitopes that are sensitive to periodate treatment, tissue sections were fixed with acetone and paraformaldehyde, exposed to hydrogen peroxide as described above, and then incubated in 0.05 mol/L sodium acetate, pH 6.0 with or without 0.02 mol/L sodium periodate for 2 hours at room temperature. After sections were washed with phosphate-buffered saline, the immunohistochemical analysis was continued as described.

To test for the presence of sialic acid-containing epitopes, tissue sections were fixed with acetone and paraformaldehyde, exposed to hydrogen peroxide as described, and then digested with *Vibrio cholerae* neuraminidase (0.1 U/ml) in phosphate-buffered saline that contained Ca^{++} and Mg^{++} at pH 7.0 for 90 minutes at 37° C. After sections were washed with phosphate-buffered saline, the immunohistochemical analysis was continued as described.

Results

The clinical characteristics of the patients, the specimens that were studied, and the histologic features of the tumors are shown in Table II. In general, the antigenic phenotypes that were observed in this study had no correlation with tumor grade and histologic cell type. Most significantly, almost no variation was seen in antigen expression among specimens that were obtained from various sites in the same patient for either the cell-surface markers or blood group markers (Tables III and IV). Intratumoral antigenic heterogeneity (variation in antigen expression from area to area with an individual tumor specimen) was observed with monoclonal antibodies that detect carbohydrate determinants, whereas antibodies that detect protein epitopes showed minor or no variation in antigen expression. Even in tumors that stain heterogeneously, however, comparison of antigenic phenotypes among primary and metastatic sites, including tumor cells from ascitic fluid, showed only minor differences in the percentage of cells that stained positive. Thus within a given patient's specimens, the antigenic phenotype was quite consistent from site to site with this antibody group. As anticipated, variations were seen in antigen expression in tumors from different patients.

All of the epithelial ovarian cancer specimens that were examined in this study expressed MH99, MW162, and epithelial membrane antigens on the majority of tumor cells. Specimens from some patients also expressed CA125, MX35, and MW207 antigens with a homogeneous staining pattern. However, areas of variable staining were noted in specimens from some patients with OC125, MX35, and MW207 antibodies; this heterogeneous staining could not be correlated with tumor grade or histologic cell type of the tumor. Periodate treatment or sialidase digestion of these tumor specimens before immunohistochemical staining also did not alter the pattern of reactivity, which suggests that the epitopes that were detected by these monoclonal antibodies were not carbohydrate in nature. In contrast, monoclonal antibody B72.3 detects a periodate-sensitive epitope. Recent studies,[6, 7] have shown that the epitope that is recognized by monoclonal antibody B72.3 is sialyl-Tn (TAG72). Five of the 12 patients who were studied had moderately high expression of TAG72 in tumors, and at least 70% of tumor cells showed a luminal pattern of reactivity. In contrast, only minor populations of cells (1% to 10%)

Table III. Expression of epithelial cell differentiation antigens

Specimen	*Antigen*								
	MH99	*T16*	*CA125*	*MW162*	*EMA*	*MX35*	*MW207*	*MT334*	*TAG72*
NA									
p	●	◒	◒	●	●	●	●	○	○
m	●	●	●	●	●	●	◒	○	○
a	●	◒	●	●	●	●	◒	○	○
LC									
p	●	◒	◒	●	◒	◒	●	○	◒
m	●	◒	◒	●	◒	◒	●	○	◒
AC									
p	●	●	●	●	●	●	●	◒	◒
m	●	●	●	●	●	●	●	○	◒
SG									
m1	●	●	●	●	●	◒	●	○	○
m2	●	●	●	●	●	◒	●	○	○
MJ									
p	◒	◒	◒	●	●	○	○	○	○'
m	●	◒	◒	●	●	◒	●	○	○
BK									
p1	●	●	●	●	●	●	●	○'	◒
p2	●	◒	●	●	◒	●	●	○	◒
m	●	◒	●	●	●	●	●	○	◒
RL									
p1	●	◒	●	●	●	◒	◒	○	◒
p2	●	◒	●	●	●	●	◒	○	◒
m	◒	●	●	●	●	●	◒	○	◒
KM									
p1	◒	◒	◒	●	●	○	◒	◒	○
p2	●	◒	◒	●	●	○	●	○	○
m	●	●	◒	●	●	◒	●	○'	○
KMc									
p1	●	◒	●	●	●	◒	●	◒	○'
m1	●	●	●	●	●	●	●	○	○'
m2	●	●	●	●	●	◒	●	○	○'
CM									
p1	◒	◒	●	●	●	◒	◒	○	◒
p2	◒	◒	●	●	●	◒	◒	○	◒
a	●	●	●	●	●	◒	◒	○	●
AO									
m1	●	◒	●	●	●	●	●	○	○
m2	●	●	●	●	●	●	●	○	○
JS									
p1	●	●	●	●	●	●	◒	○'	○
p2	◒	●	●	●	◒	●	◒	○'	○
m	●	◒	●	●	○	◒	◒	○	○

EMA, Epithelial membrane antigen; ●, homogeneous reactivity; ◒, heterogeneous reactivity with 70% to 90% cells; ◒, heterogeneous reactivity with 30% to 70% cells; ○, heterogeneous reactivity with 10% to 30% cells; ○', reactivity with <10% cells; ○, negative; *p*, primary tumor; *m*, metastatic tumor; *a*, ascites cells.

stained positive with monoclonal antibody MT334 in samples from five patients. The epitope that was detected by monoclonal antibody MT334 is a combined carbohydrate-protein structure that is carried on a mucin[8] (unpublished results).

Blood group–related antigens showed variation in expression in samples from different individuals. Three of the 12 patients had blood type A. Specimens from two of these patients retained the A blood group type of the patient, whereas in the other patient A antigen was not detected in the tumor. Nine of the 12 patients had blood type O (H). Specimens from one patient with blood type O retained strong H expression in an endometrioid-type ovarian carcinoma; H antigen expression was heterogeneous in the samples from six patients with serous or undifferentiated tumors and undetectable in the remaining two, which were samples from serous tumors. In a metastatic lesion from one patient (N.A.) with a primary tumor that stained heterogenously, loss of ABH antigen expression was observed in areas in which the tumor cells were poorly differentiated. However, in another patient (S.G.) with a poorly differentiated tumor, there was a strong homogeneous pattern of A antigen expression in both of the metastatic lesions that were tested.

Intratumoral heterogeneity was also observed with

Table IV. Expression of blood-group-related antigens

Specimen	Antigen								
	Le^a	*SLe^a*	*Le^b*	*Le^x*	*Le^y*	*Le^y/H-2*	*H-2*	*A*	*B*
NA									
p	○	○	○	◒	●	●	●	○	○
m	○	○	○	◒	●	●	◒	○	○
a	○	○	○	●	●	●	○′	○	○
LC									
p	◒	◒	○′	○	◒	◒	○′	○	○
m	◒	◒	◒	○	◒	◒	◒	○	○
AC									
p	○	○	○	○	◒	●	◒	○	○
m	○	○	○	○	◒	●	◒	○	○
SG*									
m1	○	○	○	○	○	◒	○	●	○
m2	○	○	○	○	○	◒	○	●	○
MJ									
p	○	◒	◒	◒	◒	◒	◒	○	○
m	◒	◒	●	●	◒	●	◒	○	○
BK*									
p1	○′	◒	○	◒	○	◒	○	◒	○
p2	◒	◒	○	◒	○	◒	○	◒	○
m	○′	○′	○	◒	○	◒	○	◒	○
RL*									
p1	○′	○′	○	○	◒	●	◒	○	○
p2	○′	○′	○	◒	◒	◒	○′	○	○
m	○′	◒	○	◒	◒	●	◒	○	○
KM									
p1	◒	◒	◒	○	◒	◒	●	○	○
p2	◒	◒	●	○	◒	◒	●	○	○
m	◒	◒	●	○	◒	◒	●	○	○
KMc									
p1	◒	◒	●	○	◒	◒	○	○	○
m1	◒	◒	◒	○	◒	◒	○	○	○
m2	○′	○′	●	○	○	◒	○	○	○
CM									
p1	○	○	○	○	◒	●	◒	○	○
p2	○	○	○	○	◒	●	◒	○	○
a	○	○	○	○	○	●	◒	○	○
AO									
m1	○	○	○	○	○	◒	◒	○	○
m2	○	○	○	○	◒	◒	◒	○	○
JS									
p1	◒	◒	○	○	○	◒	○	○	○
p2	◒	◒	○	○	○	○	○	○	○
m	○	○	○	○	○′	◒	○	○	○

●, Homogeneous reactivity; ◒, heterogeneous reactivity with 70% to 90% cells; ◒, heterogeneous reactivity with 30% to 70% cells; ○, heterogeneous reactivity with 10% to 30% cells; ○′, reactivity with <10% cells; ○, negative; *p*, primary tumor; *m*, metastatic tumor; *a*, ascites cells.

*The blood type of these patients was A; all others were type O.

the Lewis blood-group antigens Le^a, sialyl-Le^a, and Le^b, and histologically similar areas within a specimen showed similar patterns of reactivity. In most instances all three antibodies were either positive (3 of 12 patients) or negative (7 of 12 patients) within a specific tumor area. In two cases, only Le^a and sialyl-Le^a were detected. These results suggest the possibility that ovarian tumors could be subclassified on the basis of Lewis antigen expression, but this observation and its clinical significance require further study.

Comment

In this study we found remarkably little variation in the antigenic expression of epithelial ovarian cancer among primary and metastatic sites in the same patient with the use of a group of monoclonal antibodies to detect a spectrum of cell-surface antigens. The only exception to this observation was the loss of expression of Le^a and sialyl-Le^a in metastatic tumor from a single patient. In two patients (S.G., A.O.) no primary tumor was tested; antigen expression was essentially identical between two metastatic sites for each of these patients. These findings are consistent with previous work from this laboratory,[5] which showed no significant variation over time in the antigen expression of ovarian tumors that were sampled at sequential operations in individual patients, and confirm the findings in a recent report by Berchuck et al.[9] that employed a smaller group of

antibodies to evaluate antigen expression among primary and metastatic sites in epithelial ovarian cancer.

A number of additional observations can be made regarding antigen expression in epithelial ovarian cancer. The secretion of mucins and high molecular weight glycoproteins, such as CA125, is characteristic of epithelial ovarian cancers. Several of the antibodies that we have studied react with the carbohydrate on these mucins. For example, one antibody (B72.3) detects sialyl-N-acetylgalactosaminyl serine or threonine (i.e., O-linked chains). Others detect the blood group–related determinants Le^a, Le^b, and sialyl Le^a, which can be either O-linked or linked to asparagine (i.e., N-linked chains) in glycoproteins. The antigens A, B, and H, and Lewis antigens can also be carried on glycolipids. It is of interest that the serous papillary tumors often showed a reciprocal staining pattern between B72.3 and anti-Lewis antibodies (Tables III and IV). This observation suggests a possible explanation for one aspect of intratumoral heterogeneity and suggests that a particular tumor tends to synthesize characteristic carbohydrate structures. Nakasaki et al.[10] similarly suggested that the intratumor antigenic mosaicism that is found in colonic and gastric cancers is due to cell populations that display different degrees and types of glycosylation. In a single patient (J.S.), there appeared to be a loss of Lewis antigen expression in the metastatic tumor, which was somewhat less well differentiated than the primary tumor. Such loss of blood group antigen expression has been associated with increased invasiveness in cancers of the urinary bladder.[11]

One might have expected considerable antigenic diversity to be present in ovarian cancer specimens from different sites, given the degree of intratumoral heterogeneity that was demonstrated in this and our previous study,[5] our current concept of the spread of this cancer, and the fact that considerable variation in antigen expression has been demonstrated in other tumor systems.[10, 12-14] It is possible, of course, that differences may exist in the expression of antigens that were not examined in this study. We have, however, included a number of antibodies with patterns of reactivity on ovarian cancers and normal tissues that make them important candidates for clinical use, such as OC125, B72.3, MX35, and MH99. The consistent expression among primary and metastatic sites of the antigens that are detected by these antibodies encourages the continued search for clinical applications for monoclonal antibodies in patients with ovarian cancer.

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Reproducibility of the oral glucose tolerance test in pregnancy

Frederick E. Harlass, MD,* Kim Brady, MD, and John A. Read, MD
Tacoma, Washington

This prospective investigation evaluated the reproducibility of the 100 gm oral glucose tolerance test. Sixty-four obstetric patients with ≥135 mg/dl on the 50 gm oral glucose screening test were scheduled for the 100 gm test. All patients repeated the oral glucose tolerance test in 1 to 2 weeks. Both tests included a preparatory diet, and testing conditions were identical. There were no significant differences in the mean test values at each testing interval when the entire study population was considered. Patients were then divided into four groups according to the outcome of the two tests. Forty-eight of 64 (75%) had normal results at each testing period (group 1); 11 of 64 (17%) had initially normal results and abnormal results on retest (group 2); 3 of 64 (5%) had initially abnormal results and normal results on retest (group 3); 2 of 64 (3%) had abnormal results at both testing phases (group 4). There were no significant differences between oral glucose tolerance test results within groups 1 and 4. However, significant differences occurred within groups 2 and 3 between the two tests. Group 2 patients had a greater frequency of an abnormal 1-hour value on the test than group 1 patients ($p = 0.001$). Overall, the reproducibility of the oral glucose tolerance test was 78% (50 of 64). We recommend the oral glucose tolerance test be repeated when the 1-hour value is abnormal or when the fasting blood sugar, 1-hour, and 2-hour values are near the upper end of the normal range. (AM J OBSTET GYNECOL 1991;164:564-8.)

Key words: Oral glucose tolerance test, pregnancy, reproducibility

O'Sullivan and Mahan[1] defined the abnormal glucose tolerance test (GTT) on the basis of data obtained from 752 asymptomatic pregnant patients. With the use of the 100 gm oral glucose load, the mean and 2 SDs defined the upper limits of normal at each hourly testing period. These criteria have been shown to have physiologic significance for predicting future diabetes and perinatal morbidity and mortality.

As recommended by the Second International Workshop Conference[2] on Gestational Diabetes, all pregnant women should be screened for glucose intolerance between 24 and 28 weeks' gestation. Any patient with a venous plasma glucose value >140 mg/dl should have the full diagnostic glucose tolerance test.

In the nonpregnant population, the reproducibility of the 3-hour oral GTT has been evaluated. Rushforth et al.[3] investigated the reproducibility of the test in a population of 61 nonpregnant Pima Indians. These patients were given two oral GTTs 1 to 3 weeks apart. They found a mean difference of 30.1 mg/dl for the 1-hour value and 22.3 mg/dl for the 2-hour value of the oral GTT. Hence, there was considerble disagreement in the classification of persons as to their diabetic status. These results are consistent with other investigations reported in the literature.[4-8]

To our knowledge, the reproducibility of the oral GTT during pregnancy has not been investigated. The purpose of this prospective investigation is to determine the reproducibility of the 100 gm oral GTT in pregnancy.

Material and methods

This prospective investigation was conducted at Madigan Army Medical Center between September 1, 1987, and December 31, 1987. This institution serves a population composed principally of dependents of Army enlisted personnel. The mean family income is $11,000 to $16,000. All patients have unrestricted access to medical care, and no direct monetary payment is required for any physician or ancillary service. Madigan Army Medical Center is a tertiary care center that receives referrals from other military installations. Our obstetric population comprises 72% white, 16% black, 5% Hispanic, 5% Oriental, and 2% other patients.

All obstetric patients at Madigan Army Medical Center undergo the 1-hour, 50 gm oral glucose screening test. This test is performed at the initial obstetric visit if risk factors for gestational diabetes mellitus including age >25 years, obesity, history of macrosomia, previous stillborn fetus, or a family history of diabetes mellitus are present. In the absence of risk factors, all patients are screened at 24 to 28 weeks' gestation with the oral glucose screening test including patients who had normal test results at their initial obstetric visit.

If the threshold on the 50 gm oral glucose screening

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Madigan Army Medical Center.
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Reprint requests: Frederick E. Harlass, MD, Department of Obstetrics and Gynecology, William Beaumont Army Medical Center, El Paso, TX 79920-5001.
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Table I. Mean (±SD) glucose values on the oral GTT for each testing period

Test interval	*Test period*			*Pearson's correlation coefficient*	
	1	*2*	*p Value*	*r*	*p Value*
Fasting	90 ± 14	87 ± 10	NS	0.2	NS
1-Hour	162 ± 36	162 ± 36	NS	0.3	.001
2-Hour	138 ± 26	136 ± 28	NS	0.0	NS
3-Hour	113 ± 30	114 ± 31	NS	0.0	NS

test is ≥135 mg/dl, the patient is scheduled for a 100 gm oral GTT. The patients are requested to consume a diet of at least 150 gm of carbohydrate per day for 3 days before the test. The test preparation and performance is in accordance with the Second International Workshop Conference on Gestational Diabetes. The diagnosis of gestational diabetes mellitus is made when two or more venous plasma glucose concentrations meet or exceed the following blood sugar levels: fasting level of 105 mg/dl; 1-hour of 190 mg/dl; 2-hour of 165 mg/dl; and 3-hour of 145 mg/dl.[2] Patients who meet the criteria for gestational diabetes mellitus are admitted to the hospital for dietary or dietary and insulin management. Patients are then followed up through the complicated obstetric clinic to ensure adequate control and compliance.

Sixty-four obstetric patients who were scheduled for the oral GTT were enrolled in the investigation. A second oral GTT was performed in approximately 1 to 2 weeks. Test preparation and performance conditions were identical between the two testing periods. Fasting blood sugar levels were obtained between 8 and 9 AM. Patients were allowed to ambulate; however, they were not allowed to eat, smoke, drink any beverage other than water, or exercise. Any patient who did not tolerate the glucose solution was removed from the investigation. Patients were then divided into one of four groups, depending on the results of the two oral GTTs. The four groups were as follows: patients in group 1 had a normal oral GTT results followed by normal oral GTT results, group 2 patients had a normal results followed by abnormal results, group 3 patients had abnormal results followed by normal results, and group 4 patients had abnormal results followed by abnormal results. In addition, the reproducibility within the four groups was evaluated by the trimester in which the tests were performed.

Significance of continuous data was assessed by the Student *t* test for paired data. Statistical analysis of discrete data was performed with χ^2 analysis or Fisher's exact test. Pearson's correlation coefficient *(r)* was calculated for paired responses at each testing interval of the oral GTT with Lotus 123 (Lotus Development Corporation, Cambridge, Mass.). A probability value of <0.05 was considered significant.

Results

The mean age of the study population was 27 ± 5.6 years; 22 were nulliparous. Forty-eight of 64 (75%) patients had normal results at both testing periods (group 1). Eleven of 64 (17%) were normal at the initial period, but abnormal at repeat testing (group 2). Three of 64 (5%) patients were abnormal at the initial testing period, but normal at retesting (group 3). Two of 64 (3%) patients had abnormal results at both testing periods (group 4). Table I shows the mean oral GTT values at each interval for the entire population. There were no statistically significant differences between the two testing periods. In addition, only the 1-hour value during the two testing periods was significantly associated.

Table II shows the oral GTT values at each testing period when evaluated by groups. There was no significant difference between tests within groups 1 (normal-normal) and 4 (abnormal-abnormal). However, significant differences occurred between tests within groups 2 (normal-abnormal) and 3 (abnormal-normal). Between groups 1 (normal-normal) and 2 (normal-abnormal), there were significant differences in the mean plasma glucose values at fasting and at 1-hour and 2-hour test intervals ($p = 0.02$, $p = 0.003$, $p = 0.02$, respectively). Table III compares the number of patients in group 1 (normal-normal) with the number of patients in group 2 (normal-abnormal) who had one of four values that met or exceeded the limits on the oral GTT. Of note, 55% (6 of 11) of patients in group 2 had an abnormal 1-hour value and 72% (8 of 11) of patients in group 2 had one abnormal value compared with only 17% (8 of 48) of patients in group 1. Of the two groups (groups 3 and 4) that had abnormal initial test results, only two of five (45%) were reproducible. Moreover, 48 of 59 (81%) of patients with normal initial results had reproducible tests.

There were no significant differences between trimesters in the reproducibility of the oral GTT ($p = 0.4$). Twelve patients had the tests performed in the first trimester, all of which were reproducible. Of the 42 patients who were evaluated in the second trimester, 31 (74%) were reproducible. Seven of ten (70%) patients who were evaluated in the third trimester had reproducible tests. In our population, significantly

Table II. Mean serum oral GTT values when segregated by groups at each testing period

		Interval							
Oral GTT test period		*Fasting*				*1-hour*			
Group	*n*	*Initial*	*Repeat*	*p Value*	*r**	*Initial*	*Repeat*	*p Value*	*r*
1	48	87	85	NS	0.2	154	151	NS	0.2
2	11	97	89	NS	0	188	205	†	0.1
3	3	88	98	§	1	154	125	NS	1
4	2	111	104	NS	‖	225	220	NS	‖

*Pearson's correlation coefficient.
†$p \leq 0.01$.
‡$p \leq .001$.
§$p \leq 0.05$.
‖Too few observations to determine.

Table III. Distribution of patients with one abnormal value on the oral GTT in groups 1 and 2

Test interval	*Group 1 (normal-normal)*		*Group 2 (normal-abnormal)*		*p Value*
	Frequency	*%*	*Frequency*	*%*	
Fasting	1/48	2	1/11	9	NS
1-Hour	4/48	8	6/11	55	0.001
2-Hour	1/48	2	1/11	9	NS
3-Hour	2/48	4	0/11	0	NS
TOTAL	8/48	17	8/11	72	0.0007

more patients had normal initial results with abnormal retest results ($n = 11$) than abnormal initial results with normal retest results ($n = 3, p = 0.04$). The reason for the difference is not apparent. The difference may be a result of an α error inasmuch as there were no significant differences between the two groups in demographic data (age, parity, or weeks' gestation at each testing phase). Overall, 50 of 64 (78%) oral GTTs were reproducible (groups 1 and 4 of groups 1, 2, 3, 4). The mean birth weight of group 1 (normal-normal) was 3525 gm, which was not significantly different from group 2 (normal-abnormal, 3589 gm), group 3 (abnormal-normal, 4096 gm), and group 4 (abnormal-abnormal, 3677 gm).

Comment

The clinical value of a test depends greatly on its reliability. In addition, the diagnostic value of any test is determined by its ability to discriminate normal from abnormal in comparison to its susceptibility to intraindividual variation. It is well recognized that gestational diabetes mellitus is associated with an increased incidence of maternal and fetal complications. Therefore the accurate diagnosis of gestational diabetes mellitus is important. In both pregnant and nonpregnant populations, the oral GTT has represented the cornerstone in the diagnosis of gestational diabetes mellitus. Unfortunately, there is no "gold standard" by which to judge the accuracy of the oral GTT.

Troxler et al.[9] investigated the oral GTT by 11 different criteria in a nonpregnant population and was able to show a reproducibility of only 50%. McDonald et al.[6] performed six 100-gm, 3-hour oral GTTs on 334 asymptomatic male volunteers. They documented a variation of ±34 mg/dl at the 1-hour interval over the six tests. Among the six tests, a number of patients varied from normal test results to having a test diagnostic of diabetes mellitus. In a related investigation on the reproducibility of the 50-gm, 1-hour glucose screening test, Sacks et al.[10] found that the screening test was only "moderately reproducible." With the use of a single screening test with the test value ≤135 mg/dl, they would have missed 27% of the diabetic population. Because of the marked variability that may be encountered in oral GTTs in the nonpregnant population, Nelson[11] has stated that unless the test is grossly abnormal, it should be performed on two occasions before diabetes mellitus is diagnosed.

In most populations, plasma glucose values are continuous measurements with a unimodal distribution. Irrespective of the values chosen for the limit of normal on the oral GTT, some patients with gestational diabetes mellitus will be falsely classified as normal and others without gestational diabetes mellitus will be misclassified as having gestational diabetes mellitus. If one considers that it is more important to overdiagnose gestational diabetes mellitus than to not diagnose the disease when it is present, the important group to consider

Interval							
2-Hour				3-Hour			
Initial	*Repeat*	*p Value*	*r*	*Initial*	*Repeat*	*p Value*	*r*
131	129	NS	0	110	107	NS	0
147	169	‡	0	111	145	‡	0.1
189	105	‡	1	182	97	‡	1
185	172	NS	‖	138	129	NS	‖

in our investigation is group 2 (e.g., those who had normal initial oral GTT results, but on retesting had abnormal results). In this clinical situation, the patient would be followed-up more closely with diet therapy (with or without insulin) and fetal heart rate testing. However, if one considers that it is more important to NOT overdiagnose gestational diabetes mellitus, the important population to consider is group 3 (e.g., those who had abnormal initial oral GTT results, but on retesting had normal results). Most clinicians would rather risk overdiagnosis of gestational diabetes mellitus and provide the patient with closer surveillance.

The mean plasma oral GTT values for groups 1 and 2 were within the normal range. However, our investigation showed significant differences in the mean plasma glucose values at the fasting, one-hour, and two-hour intervals on the oral GTT in those patients who had normal oral GTT results followed by abnormal oral GTT results (group 2) when compared with patients with two normal oral GTT results (group 1). Because the majority of laboratories use the glucose oxidase method to measure plasma glucose, Carpenter and Coustan[12, 13] recommend use of a fasting value of 95 mg/dl, a 1-hour value of 180 mg/dl, a 2-hour value of 155 mg/dl, and a 3-hour value of 140 mg/dl as representative of the threshold values of the oral GTT. In addition, a recent investigation by Sacks et al.[14] further supports the concept that the currently accepted values on the oral GTT may be too high. They recommend a fasting threshold value of 96 mg/dl, a 1-hour value of 172 mg/dl, a 2-hour value of 152 mg/dl, and a 3-hour value of 131 mg/dl. These values agree with Carpenter and Coustan's values and the values (mean ± 1 SD) we obtained in group 1 patients.

Significantly more patients in study group 2 (normal-abnormal) had one abnormal oral GTT value than in group 1 (normal-normal) patients (8 of 11 versus 8 of 48, respectively, $p = 0.0006$). The most frequent abnormal value on the oral GTT was the 1-hour value (Table III). Ten of 59 (17%) patients (groups 1 and 2) had initially normal oral GTT results with an abnormal 1-hour value. On repeat testing, 6 of the 10 patients had oral GTT results diagnostic of gestational diabetes mellitus. The mean birth weight of infants born to mothers in groups 2, 3, and 4 was not significantly different from those in group 1. We believe this is attributable to therapeutic intervention of group 2 (diet therapy and insulin if necessary).

The results of our investigation show a significant variability in oral GTT results when glucose values are in the upper-normal range. In many cases, this variability was sufficiently large to reclassify the patient as having gestational diabetes mellitus. In summary, we recommend the oral GTT be repeated if the patient has an abnormal 1-hour value or the fasting, 1-hour, and 2-hour oral GTT values are normal but elevated.

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Steroid-responsive nephrotic syndrome with renal insufficiency in the first trimester of pregnancy

Luis G. Uribe, MD, Vashu D. Thakur, MD, and N. Kevin Krane, MD
New Orleans, Louisiana

Successful treatment of a woman with renal failure due to presumed minimal change nephropathy in the first trimester of pregnancy is presented. (Am J Obstet Gynecol 1991;164:568-9.)

Key words: Nephrotic syndrome, pregnancy, renal insufficiency

We report a case of renal insufficiency and severe nephrotic syndrome that was diagnosed and treated in the first trimester of a woman who was first seen with severe edema. Minimal change nephropathy was the presumptive diagnosis, and although this entity has been reported in pregnancy, its presentation with renal failure makes this a particularly unusual case.[1]

A 22-year-old black woman, gravida 2, para 1, abortus 0, was seen at 18 weeks' gestation with a 2-day history of swelling in her legs and a 3-day history of influenza-like symptoms for which she was given penicillin. The patient had a history of nephrotic syndrome since age 5 years and had received prednisone therapy on at least three occasions, the last course being 2 years before this admission to the hospital. During her most recent relapse, the patient had hypertension, edema, and a rise in her serum creatinine level, all of which resolved with steroid therapy.

On physical examination, the blood pressure was 110/70 mm Hg, fundal height was consistent with 18 weeks' gestation, and there was 2 to 3+ pitting edema up to the knees. The white blood cell count was 5200/mm^3, hemoglobin 9.5 gm/dl, hematocrit 32.1%, and platelet count 702,000. The urine dipstick showed 4+ proteinuria, with 5.5 gm of protein and 1.31 gm of creatinine in a 24-hour urine specimen. The serum creatinine level was 2.2 mg/dl, and the electrolyte values were sodium 125 mEq/L, potassium, 4 mEq/L; chloride, 100 mEq/L; carbon dioxide, 2-19 mEq/L. The blood glucose level was 75 mg/dl. The total protein was 3.8 mg/dl, serum albumin 0.9 mg/dl, and uric acid 6.2 mg/dl. An ultrasonographic examination of the kidneys showed normal-sized kidneys with no obstruction. Serologic studies for secondary causes of nephrotic syndrome, including hepatitis, syphilis, and lupus, were negative.

In view of the previous history of steroid-responsive nephrotic syndrome, a presumptive diagnosis of minimal-change nephropathy was made and the patient was started on 20 mg prednisone three times a day. Within a week of steroid therapy, the urine dipstick became negative, edema had decreased considerably, and serum creatinine levels had fallen to 0.6 mg/dl. Over the next 2 months the 24-hour urine excretion fell to 100 mg/24 hours and the prednisone was discontinued with no relapse. She was delivered of a 1960 gm boy at 35 weeks' gestation with Apgar scores of 9 at 1 and 5 minutes.

Comment

Renal failure during pregnancy presents an unusual challenge in diagnosis and management to the obstetrician. Whereas there are many causes of acute renal failure during pregnancy, the urinalysis provides the key in the evaluation of acute renal failure in the first two trimesters of pregnancy.[2] When this patient was first seen with edema, the urinalysis showed significant proteinuria, and a 24-hour urine collection confirmed the diagnosis of nephrotic syndrome. In general, the approach to patients with nephrotic syndrome depends heavily on the history and physical examination when trying to decide which patients have primary or

From the Department of Medicine, Section of Nephrology, Tulane University School of Medicine.
Received for publication June 17, 1990; revised September 25, 1990; accepted October 24, 1990.
Reprint requests: N. Kevin Krane, MD, Tulane University School of Medicine, 1430 Tulane Ave., New Orleans, LA 70112.
6/1/26278

idiopathic forms of the nephrotic syndrome, or a secondary type associated with a systemic disease such as systemic lupus erythematosus, diabetes mellitus, or preeclampsia.

The history in this patient provided the key to the diagnosis. Because she was seen in early pregnancy without hypertension made preeclampsia unlikely, and there were no findings to suggest either diabetes or systemic lupus erythematosus. She had a clear history of steroid-responsive nephrotic syndrome beginning in childhood, typical of minimal-change nephropathy. Her history of explosive onset of severe nephrotic syndrome is also characteristic of this disorder. Acute renal failure is not typically associated with minimal-change nephropathy; however, this complication has been well described, particularly in adults with this disease. Although the diagnosis of this disease can be established with certainty only by renal biopsy, it was believed that the presentation was so typical of this disease that a therapeutic trial of corticosteroids was the safest approach. Rapid response to treatment with complete resolution of nephrotic syndrome and renal failure supported the diagnosis. If there had been no response to therapy, then a renal biopsy to exclude other disorders would have been appropriate.

This patient who was seen with renal failure in early pregnancy illustrates the importance of a systematic approach to this problem. By making a presumptive diagnosis of nephrotic syndrome as a result of minimal-change disease on the basis of her clinical presentation, therapy with prednisone led to an uneventful pregnancy with successful delivery.

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Pharmacokinetics of acyclovir in the term human pregnancy and neonate

Lisa M. Frenkel, MD,[a] Zane A. Brown, MD,[b] Yvonne J. Bryson, MD,[a] Lawrence Corey, MD,[c] Jashvant D. Unadkat, PhD,[d] Paul A. Hensleigh, MD, PhD,[e] Ann M. Arvin, MD,[f] Charles G. Prober, MD,[f] and James D. Connor, MD[g]

Los Angeles, Stanford, and San Diego, California, and Seattle, Washington

Concern about neonatal herpes often leads to cesarean delivery of infants in women with a history of genital herpes. The antiviral drug acyclovir has been used effectively to suppress genital herpes simplex virus recurrences in nonpregnant adults. Its administration to pregnant women with recurrent genital herpes may reduce herpes simplex virus recurrences and thus may decrease the cesarean section rate among this population. To study the pharmacokinetics, safety, and patient tolerance of suppressive oral acyclovir, either 200 mg (n = 7) or 400 mg (n = 8) was administered orally every 8 hours to pregnant women with a history of recurrent herpes simplex virus, from 38 weeks' gestation until delivery. The mean ± SD plasma levels for the 200 and 400 mg groups, respectively, were: first dose peak, 1.7 ± 0.6 and 2.3 ± 1.0 μmol/L; steady-state trough, 0.7 ± 0.3 and 0.8 ± 0.6 μmol/L; steady-state peak, 1.9 ± 1.0 and 3.3 ± 1.0 μmol/L. In late gestation maternal acyclovir pharmacokinetics were similar to those of nonpregnant adults from other studies. Acyclovir was concentrated in the amniotic fluid; however, there was no accumulation in the fetus (mean maternal/infant plasma ratio at delivery was 1.3). Acyclovir was well tolerated, and no toxicity was seen in the mothers or infants. The administration of acyclovir, 400 mg every 8 hours, appears appropriate for use in an efficacy and safety study regarding suppression of herpes simplex virus recurrences during the last weeks of pregnancy. (Am J Obstet Gynecol 1991;164:569-76.)

Key words: Genital herpes in pregnancy, acyclovir pharmacokinetics

From the Division of Infectious Diseases, Department of Pediatrics, University of California at Los Angele Center for Health Sciences,[a] the Departments of Obstetrics and Gynecology,[b] Medical Virology,[c] and Pharmaceutics,[d] University of Washington at Seattle, the Department of Obstetrics and Gynecology[e] and the Division of Infectious Diseases, Department of Pediatrics,[f] Stanford University, and the Division of Infectious Diseases, Department of Pediatrics, University of California at San Diego.[g]

Received for publication March 27, 1990; revised August 3, 1990; accepted August 31, 1990.
Reprint requests: Lisa M. Frenkel, MD, Assistant Professor of Pediatrics in Residence, UCLA Center for Health Sciences, 10833 LeConte Avenue, Room 22-442 MDCC, Los Angeles, CA 90024-1752.
6/1/25065

Table I. Demographic characteristics of mother and newborn

	Acyclovir 200 mg t.i.d.						
	Patient No. 1	*Patient No. 2*	*Patient No. 3*	*Patient No. 4*	*Patient No. 5*	*Patient No. 6*	*Patient No. 7*
Age	29	30	38	29	30	29	35
Weight	65	82	72	62	70	84	89
No. of days of acyclovir	10	7	6	11	15	10	16
No. of recurrences, this pregnancy	10	2	5	1	8	3	3
Labor and delivery complications	Yes*	No†	Yes‡	No	No	Yes§	Yes‖
Delivery route	Vag.	Vag.	Vag.	Vag.	Vag.	C/S	C/S
Apgar score (1 and 5 min)	9/9	9/9	9/9	9/10	8/8	8/9	8/9
Sex	M	F	F	M	M	M	F
Weight (gm)	3263	3575	3234	3405	3459	3902	3602
Newborn complications	No	No	Yes††	No	No	No	No
Normal at 6 mo	Yes	Yes	Yes	Yes	Yes	Yes	Yes

The demographic characteristics of the mothers and infants include a mean maternal age of 31.7 years and mean weight of 74 kg. Several minor complications occurred during labor and delivery.

*Retained placenta with postpartum bleeding.

†Meconium staining of amniotic fluid.

‡Approximately 10% abruptio placentae.

§Cephalopelvic disproportion.

‖Failure of labor to progress.

¶Umbilical cord prolapse.

#HSV.

**Breech presentation.

††Delayed closure of ductus arteriosus.

Although neonatal herpes simplex virus (HSV) infections are not routinely reported to public health officials, studies from the state of Washington showed an increase in the incidence of neonatal herpes from 2.6 per 100,000 in 1969 to 11.9 per 100,000 in 1981[1] and to 15.4 per 100,000 from 1982 to 1985.[2] This increase probably occurred as the result of an increased prevalence of genital HSV infection in the population of childbearing age, as suggested by a nationwide 7.5-fold increase in private physician consultations for genital herpes from 1966 to 1981[3] and a similar increase in the incidence of genital herpes found in Rochester, Minnesota from 1965 to 1979.[4]

A cesarean section rate in excess of 50% has been reported for patients entering pregnancy with a history of symptomatic recurrent genital herpes, and is attributed in part to reactivation of HSV within 1 week of the onset of labor.[5] A substantial proportion (25%) of these cesarean sections has been assessed to be unnecessary[6] and to represent unwarranted risks, morbidity, and cost. In addition to the number of unnecessary cesarean deliveries, it is also disconcerting that cesarean section, even when performed before rupture of membranes, may not prevent transmission of HSV from the maternal birth canal to the infant.[7]

Acyclovir (Zovirax; Burroughs Wellcome, Research Triangle Park, N.C.), an antiviral agent, is activated selectively in HSV-infected cells and has been found to be safe and effective in the treatment of HSV infections, as well as in suppression of HSV recurrences in nonpregnant adults.[8-15] Acyclovir, taken orally in late pregnancy to supress reactivation of HSV, could reduce the high rate of cesarean section and the risk of HSV transmission to the infants of women with recurrent HSV infection. The appropriate dose and safety of acyclovir for use in human pregnancy have not been established. We studied the pharmacokinetics and safety of oral acyclovir in a group of pregnant women with recurrent genital HSV.

Study design and methods

Subjects were enrolled into this study at three medical centers: UCLA Medical Center in Los Angeles, the University of Washington in Seattle, and Stanford University Medical Center in Stanford, Calif. The protocol was approved by each institution's human subjects protection committee. After giving informed consent, subjects were given a screening interview and a physical examination. Only subjects with previous culture-documented genital HSV type 2 infection and a history of active recurrent genital lesions antedating and during pregnancy were included. Women with high-risk

Acyclovir 400 mg t.i.d.							
Patient No. 1	*Patient No. 2*	*Patient No. 3*	*Patient No. 4*	*Patient No. 5*	*Patient No. 6*	*Patient No. 7*	*Patient No. 8*
33	30	27	37	28	32	37	39
69	81	65	60	88	65	76	84
29	3	6	22	14	15	8	11
1	4	2	3	3	4	5	1
Yes§ C/S	No C/S#	No Vag.	Yes¶ C/S	No Vag.	No C/S**	No Vag.	Yes§ C/S
8/9	9/9	8/9	9/9	8/9	8/9	8/9	9/9
F	M	F	M	M	M	F	F
4404	3432	3253	4146	3720	3600	3540	4090
No	No	No	No	No	No	No	No
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

pregnancies or active genital herpes lesions at the time of enrollment were excluded. Normal test results for complete blood count and differential cell count, serum creatinine, blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, bilirubin, and urinalysis were required for enrollment. Acyclovir was administered to 14 healthy women, beginning at 38 weeks' gestation until delivery, during 15 pregnancies. The first seven enrollees were given acyclovir at the dose recommended in the package insert for initiation of suppressive therapy (200 mg orally, every 8 hours).

Because acyclovir is erratically absorbed and because 200 mg acyclovir is not universally effective in suppressing clinical genital herpes, a subsequent group of eight subjects was studied at a dose of 400 mg administered every 8 hours. After the first dose of acyclovir, plasma of the patients was obtained to determine the peak value. Subjects were seen biweekly during the study period for cervical and vulvar HSV cultures and steady-state peak and trough acyclovir levels. In early labor the vulva and cervix were inspected and cultured for HSV. At delivery, maternal and cord blood were collected for determination of acyclovir concentrations, hematologic results, and blood chemistry values. In selected cases amniotic fluid, gastric aspirates of the infant, colostrum, and placental tissue were obtained for determination of acyclovir levels. The nasopharynx and rectum of the infants were cultured for HSV at birth and between 48 and 72 hours of life. If a fetal monitor was used, the site was also cultured. Maternal and infant blood samples were obtained in heparinized tubes at 1.5, 3, 6, 9, 12, 24, and 48 hours post partum for acyclovir determination of plasma levels. Acyclovir concentrations were determined by radioimmunoassay,[16] in the laboratory of James Connor, MD, at the University of California, San Diego. In addition to routine postpartum care, the well-being of the mothers and children at 6 months post partum was determined by physical examination and by telephone interviews with the patients or with their health care providers.

Results

Clinical and laboratory parameters of mothers and newborns (Table I). The mothers enrolled in our study were from 27 to 39 (mean, 31.7) years old, mostly (13/14) white, gravida 1 to 4 (mean, 2.1), para 0 to 1 (mean, 0.2), and had had recognized genital herpes for 1 to 10 years (mean, 4.4). During the pregnancies studied, these women had one to 10 (mean, 3.7) HSV recurrences. The acyclovir courses ranged between 3 and 29 (mean, 13.7) days' duration. Acyclovir was well tolerated by the mothers; there were no complaints of nausea, vomiting, headache, or other symptoms, and in all cases the complete blood count with differential cell count, alanine aminotransferase, aspartate aminotransferase, bilirubin, creatinine, blood urea nitrogen, and urinalysis (including no crystaluria) were normal on days 1, 6, and 11 or the last day of therapy). At birth, all infants appeared normal and were given Apgar scores ≥8. The cord blood and newborn complete blood count with differential cell count, alanine aminotransferase, aspartate aminotransferase, bilirubin, creatinine, blood urea nitrogen, and urinalysis values were normal. Except for a delay in the closure of the ductus arteriosus of one infant, there were no postnatal complications, and at 6 months of age all infants were reportedly normal.

Viral cultures and recurrences of genital HSV. No symptomatic HSV recurrences or asymptomatic viral shedding was noted in14 of the 15 pregnancies monitored during the study period. One woman (patient 2, who was taking 400 mg of acyclovir) entered the study

Table II. Peripartum acyclovir levels

	Acyclovir 200 mg t.i.d.						
	Patient No. 1	*Patient No. 2*	*Patient No. 3*	*Patient No. 4*	*Patient No. 5*	*Patient No. 6*	*Patient No. 7*
No. of days of acyclovir	10	7	6	11	15	10	16
Maternal plasma at delivery (μmol/L)	1.09	1.70	0.65	0.76	0.80	3.51	1.09
Cord plasma (μmol/L)	1.08	1.23	0.59	0.46	0.67	2.23	1.02
Maternal/cord plasma	1.90	1.38	1.10	1.60	1.19	1.57	1.07
Amniotic fluid (μmol/L)	—	3.42	1.87	6.06	—	—	—
Gastric aspirate (μmol/L)	—	4.00	4.37	5.31	—	—	—
Placenta (μmol/L)	1.31	6.19	1.34	1.08	—	—	—
Breast milk (μmol/L)	—	—	—	—	—	—	—

Peripartum acyclovir levels for all subjects are shown (1 μmol/L = 0.225 μg/ml). Maternal acyclovir levels at time of birth were greater than those of infants (except for subject 6 of the 400 mg group), even though amniotic fluid levels were concentrated by a factor of 3 to 6. Breast milk samples were collected 4*(a)*, 3*(b)*, and 5*(c)* days after last acyclovir dose.

with a recently healed perianal HSV lesion. Culture on day 1 was negative for HSV. On the day after initiation of acyclovir (day 2), after two doses, HSV antigen was detected by an enzyme-linked immunosorbent assay on a specimen obtained from the site of the healed lesion. Labor began the next day, and the infant was delivered by cesarean section before rupture of membranes. Subsequently, a culture obtained on day 2 from the healed lesion site became positive for HSV. All cultures from this infant were negative for HSV, as were all the cultures from the other infants.

Acyclovir pharmacokinetics. Plasma acyclovir concentrations obtained after the first doses are shown in Fig. 1, *A*. The peak acyclovir plasma levels occurred between 1½ and 3 hours after the dose was administered. The mean ± SD acyclovir plasma levels at 1½ hours for the 200 and 400 mg dose groups were 1.7 ± 0.6 and 2.3 ± 1.0 μmol/L, respectively. One subject in the 200 mg dose group appeared to absorb acyclovir unusually well. In this patient acyclovir levels were evaluated by a statistical technique that tests extreme observations in a sample.[17] Her 1.5-hour value rose above the 95th percentile of the sampling distribution and therefore was excluded from the data analysis. The mean acyclovir plasma levels for the 200 and 400 mg doses, respectively, were 1.4 ± 1.3 and 2.4 ± 0.9, at 3 hours after the dose and 0.5 ± 0.1 and 1.0 ± 0.5 μmol/L at 6 hours after the dose.

Plasma acyclovir trough and peak concentrations obtained on days 6 and 11 were similar and are shown in a combined fashion in Fig. 1, *B*. The mean and SD plasma trough values of acyclovir in the 200 and 400 mg dose groups were 0.7 ± 0.3 and 0.8 ± 0.6 μmol/L, respectively. One subject in the 400 mg group took the drug three times daily instead of every 8 hours. This was reflected in high trough levels, which were determined 3 and 4 hours aftrer the previous dose. These values were excluded from the mean and SD calculation. During steady state, the 400 mg dose group's mean peak level (3.3 ± 1.0 μmol/L) was increased, as compared with the first-dose mean peak level (2.3 ± 1.1 μmol/L), whereas the suggestion of acyclovir accumulation was not seen during steady state in the 200 mg dose group with a steady-state mean acyclovir peak of 1.9 ± 1.0 μmol/L and a mean first-dose peak of 1.7 ± 0.6 μmol/L.

The peridelivery acyclovir levels are shown in Table II. The cord plasma acyclovir levels were less than the maternal acyclovir plasma levels that were obtained at the same time in all but one maternal-infant pair. In this latter case the mother's plasma acyclovir level of 0.7 μmol/L was low and was not significantly different from her infant's level of 0.8 μmol/L. The mean ratio of the maternal/cord acyclovir plasma levels was 1.3 ± 0.3. Acyclovir concentrations in the amniotic fluid (1.87 to 15.5 μmol/L, $n = 6$) were found to be three to six times higher than the corresponding cord plasma levels. Not surprisingly, gastric aspirates from the newborns ($n = 3$) had acyclovir levels similar to those of the amniotic fluid. At 3 to 5 days after the last dose of acyclovir was administered, low concentrations of the drug ($n = 2$) were detectable in breast milk. Also urine of the newborns that was collected between 12 hours and 3 days after the last dose of acyclovir had acyclovir levels between 7 and 140 μmol/L (data not shown).

The maternal plasma acyclovir levels obtained immediately before to 2 days after the last dose of acyclovir are shown in Fig. 2, together with the newborn

Acyclovir 400 mg t.i.d.							
Patient No. 1	*Patient No. 2*	*Patient No. 3*	*Patient No. 4*	*Patient No. 5*	*Patient No. 6*	*Patient No. 7*	*Patient No. 8*
29	3	6	22	14	15	8	11
—	—	—	4.10	0.50	0.67	1.70	2.20
3.36	0.84	2.82	2.90	0.00	0.81	1.50	1.90
—	—	—	1.41	∞	0.83	1.13	1.16
—	—	4.20	11.4	—	—	—	15.5
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	0.21[a] 0.22[c]	0.09[b]	—	—	—	—	—

levels obtained from birth to 2 days after the last maternal dose of acyclovir. The values are plotted from the time since the last dose of acyclovir, not in relation to the time of parturition. The mean maternal (solid line) and newborn (dashed line) acyclovir plasma values are shown. The mean maternal acyclovir plasma level is higher than that obtained from the newborn shortly after the last dose of acyclovir. However, the maternal mean acyclovir level dropped more rapidly that that of the newborn, although maternal and infant levels were similarly low by 16 to 48 hours after the last dose of acyclovir.

Comment

This study shows that acyclovir, taken orally at doses of 600 to 1200 mg/day for 3 to 29 days, was well tolerated by women late in pregnancy and did not accumulate or cause adverse effects in their newborn infants. Pharmacokinetic values of acyclovir in the pregnant women studied were similar to values of nonpregnant adults, although these comparisons are made among a limited number of subjects. First-dose oral acyclovir peak values are not published for the 200 mg oral dose in nonpregnant humans; however, M.R. Blum, PhD, of Burroughs Wellcome, provided computer-simulated values for our dosage regimens from data compiled from trials conducted among nonpregnant subjects. The Burroughs Wellcome computer-projected plasma peak for a healthy adult who ingests 200 mg of acyclovir is 1.7 μmol/L, which is identical to our mean value of 1.7 ± 0.6 μmol/L. A first-dose mean acyclovir plasma peak in nonpregnant, healthy subjects who took 400 mg for a study of acyclovir suppression of recurrent genital herpes[15] was similar to that of our pregnant women and to the Burroughs Wellcome computed value (2.3 ± 0.4 vs 2.3 ± 1.0 vs 2.6, respectively). The pregnant women's steady-state acyclovir levels for the dose of 200 mg every 8 hours compared favorably with levels of nonpregnant adults[15] and with the Burroughs Wellcome computer-generated values (mean plasma trough levels of 0.7 ± 0.3 vs 0.7 ± 0.1 vs 0.4 μmol/L and peak levels of 1.9 ± 1.0 vs 2.0 ± 0.2 vs 2.0 μmol/L, respectively). No steady-state acyclovir values for 400 mg taken every 8 hours by nonpregnant humans have been published; however, our pregnant women's values were similar to the Burroughs Wellcome computed values with mean plasma trough values of 0.8 ± 0.6 versus 0.6 μmol/L and peak values of 3.3 ± 1.0 versus 3.0 μmol/L, respectively. The large standard deviations observed were not unexpected because acyclovir has a low bioavailability and is variably absorbed.[18]

The increased blood volume of pregnant women and hence the theoretical volume of distribution of acyclovir, coupled with an increased cardiac output and concomitant renal blood flow in late gestation, suggest that acyclovir levels may be less in pregnancy than in the nonpregnant state. Surprisingly, this was not observed among our population.

Acyclovir is excreted by glomerular filtration and tubular secretion.[18] The excretion of acyclovir by the pregnant women studied appeared normal and was demonstrated both by trough values similar to those of nonpregnant humans with normal renal function[15] and by the similarity in the day 6 and day 11 trough acyclovir values.

Acyclovir was concentrated in the amniotic fluid, presumably by excretion of acyclovir in the fetal urine. Approximately 85% to 95% of acyclovir is excreted unchanged in the urine.[19] The high levels of acyclovir in the amniotic fluid may provide a pharmacologic barrier for ascending or transamniotic membrane HSV infection. No accumulation of acyclovir was observed in the fetuses of the pregnant women studied. This is

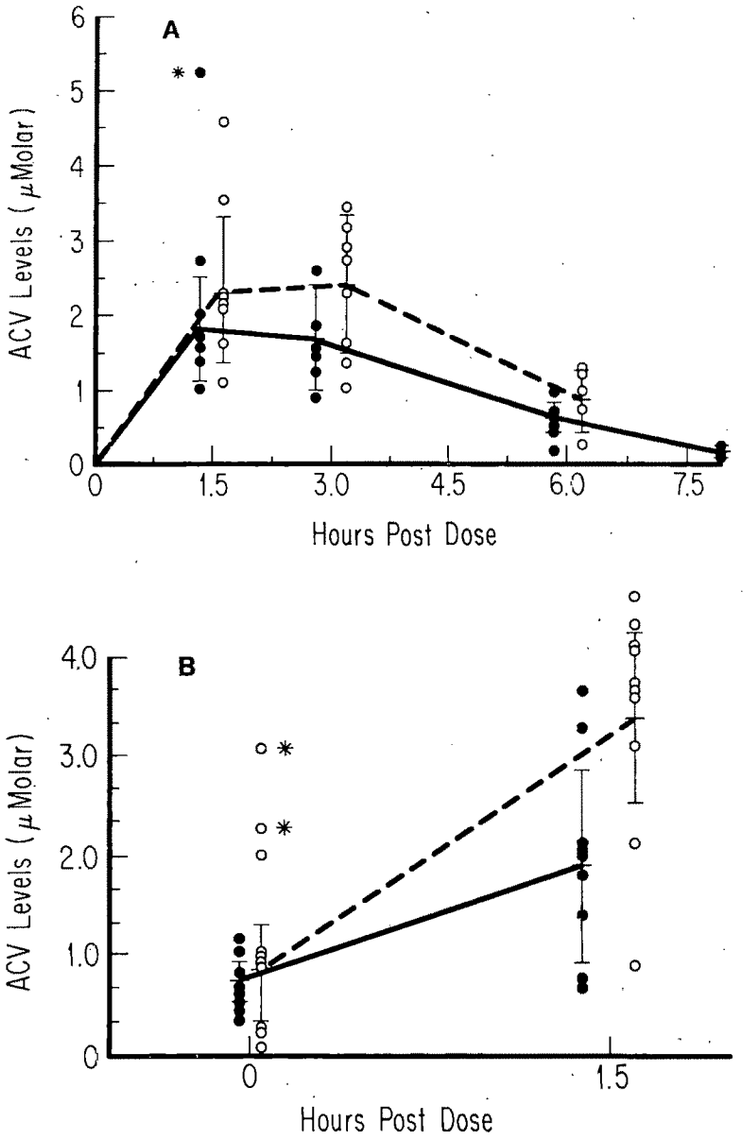


Fig. 1. Maternal acyclovir plasma levels on day 1 **(A)** and days 6 and 11 **(B).** Maternal acyclovir levels are shown as follows: **A,** first dose of 200 mg (●) and 400 mg (○). One subject *(asterisk)* absorbed the 200 mg dose of acyclovir unusually well for unclear reasons, her day 1, hour 1.5 value was above the 95th percentile of the sampling distribution for the group and therefore was excluded from the calculation of the mean and SD. Steady-state maternal acyclovir levels measured on day 6 and day 11 were similar, and are shown combined **(B).** One subject *(asterisk)* took 200 mg acyclovir three times daily instead of every 8 hours, and her elevated troughs are not included in the trough mean and SD. The 400 mg group's mean acyclovir steady-state peak of 3.3 ± 1.0 μmol/L shows accumulation of acyclovir, as compared with the day 1 mean peak of 2.3 ± 1.0 μmol/L, whereas the 200 mg day 1 and steady-state mean acyclovir plasma levels were similar, with values of 1.7 ± 0.6 and 1.9 ± 1.0 μmol/L, respectively.

an important observation, because very high acyclovir levels in the fetus could potentially cause an obstructive uropathy. A uropathy may occur when the acyclovir concentration in the renal tubules exceeds its solubility and crystallizes in the renal tubules and/or collecting ducts.[19] Glomerular filtration in the neonatal (and presumably in the fetal) kidney is impaired as compared with glomerular filtration in the adult kidney and is manifest in neonates by slower excretion of intravenous acyclovir as compared with excretion in adults.[20] Our data fail to demonstrate fetal accumulation of acyclovir, which suggests acyclovir equilibration across the pla-

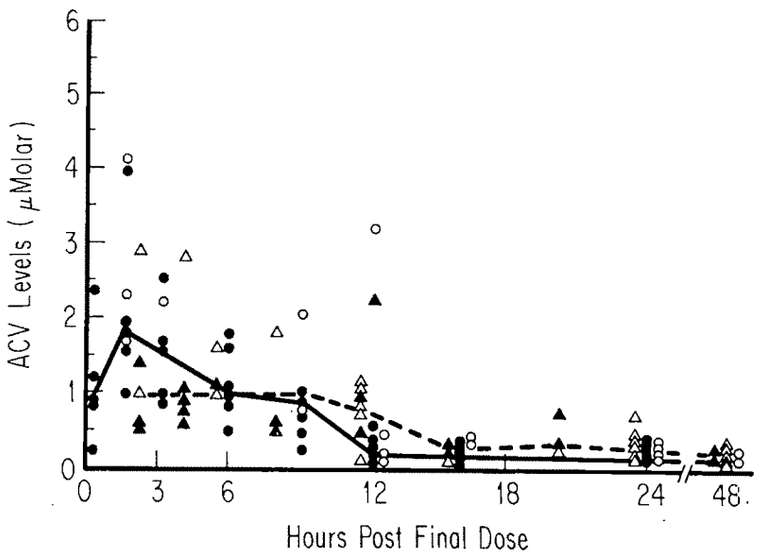


Fig. 2. Peripartum maternal and newborn acyclovir levels. Maternal and newborn plasma acyclovir levels are shown for 200 mg dose (●, mother; ▲, infant) and the 400 mg dose (○, mother; △, infant) as related to time since last maternal acyclovir dose. Delivery occurred at variable times after final acyclovir dose. *Lines* plot mean acyclovir plasma values for mother (——) and infant (- - - -) groups. Mothers had slightly higher plasma levels than did their infants soon after the last dose, but between 3 and 16 hours after the dose the mothers were noted to have excreted the acyclovir more rapidly than the newborns did.

centa. Within several hours of birth the decreased excretion of acyclovir by the neonatal kidney is observed, because the mean neonatal acyclovir plasma level exceeds that of the mothers (Fig. 2). However, by 16 hours after the last maternal acyclovir dose, both maternal and infant acyclovir levels are negligible.

Accumulation of acyclovir in breast milk has been reported.[21] Our breast milk data are sparse and do not examine peak acyclovir levels; however, as early as 2 days after acyclovir treatment is stopped, breast milk levels are negligible.

Of major concern is the safety of acyclovir treatment in the fetus. In animals, acyclovir has been studied in numerous toxicology, teratogenicity, and carcinogenicity studies.[22-27] The principal toxic effect is obstructive neuropathy, which generally ocurs with drug levels in excess of those used clinically.[24, 25] Mutagenicity has been seen in only three of more than 25 studies.[22, 25] No teratogenicity was seen in nine studies using three animal species that were given acyclovir in therapeutic doses (peak plasma levels, 1.1 to 8.6 μmol/L).[27] However, when acyclovir was given to pregnant rats in toxic doses (peak plasma levels, 440 ± 96 μmol/L) teratogenicity was observed, as was renal failure in the rat mothers.[26]

The exposure to acyclovir in this study began at 38 weeks' gestation. Since 1980 acyclovir has been given to neonates, including many premature infants, for treatment of herpes simplex infections. Thus far, no long-term effects have been identifed (Kirk E, MD, Burroughs Wellcome. Personal communication).

Burroughs Wellcome maintains a prospective registry of exposure to acyclovir in pregnancy, most of which took place during the first trimester.[28] Kenneth Lyons Jones, MD, at the University of California, San Diego, has a prospective registry of exposures to many substances, including acyclovir, during pregnancy. To date, these registries include follow-up of 112 pregnancies, and no increase or patterns of abnormalities have been observed as compared with pregnancies in which the fetuses were unexposed[26] (Jones K, MD, University of California, San Diego. Personal communication). The safety of acyclovir in early pregnancy is especially important with respect to its potential therapeutic use in the treatment of primary genital HSV infection during pregnancy, which may cause significant morbidity to both mother and infant.[29, 30]

The results of this study are reassuring, in that administration of acyclovir appeared safe for both mother and fetus when taken orally in late pregnancy. Especially noteworthy is the fact that acyclovir did not accumulate in the fetus at the doses used, in spite of its accumulation in the amniotic fluid. However, evaluation of the absolute safety of acyclovir in pregnancy will require a large, long-term, prospective, controlled (or case-controlled) study. Factors related to the morbidity of recurrent HSV infection in pregnancy are multiple and include not only infection of the infant but also an

increased rate of cesarean section with its risks and complications, the psychologic stress to the woman, and the difficulties in medical management associated with unpredictable, asymptomatic shedding of HSV from the cervix-vulva. Acyclovir treatment in the last part of pregnancy may positively affect these issues. However, a recent study of Straus et al.[31] suggests that asymptomatic shedding of genital herpes may not be decreased by suppressive acyclovir. Therefore we believe that a study to evaluate the safety and efficacy of oral acyclovir in suppressing recurrences and asymptomatic shedding of genital herpes in late gestation is warranted.

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Ovarian neoplasms and the risk of adnexal torsion

Marianne Sommerville, MD, David A. Grimes, MD, Paul P. Koonings, MD, and Keith Campbell, MD
Los Angeles, California

Previous reports examining adnexal torsion suggested that benign ovarian neoplasms are more likely to undergo torsion than malignant ovarian neoplasms. To our knowledge, this clinical observation has not been quantified. To examine this hypothesis, we reviewed our experience with ovarian neoplasms found at the time of surgery over a 10-year period. Benign ovarian neoplasms had a 12.9-fold increased risk of undergoing adnexal torsion when compared with malignant ovarian neoplasms (95% confidence interval, 10.2 to 15.9). The histologic type of ovarian neoplasm does not appear to affect the rate of adnexal torsion. Adnexal torsion rarely involves cancer. (AM J OBSTET GYNECOL 1991;164:577-8.)

Key words: Torsion, ovarian neoplasms

The most common antecedent of adnexal torsion is a benign ovarian neoplasm.[1, 2] However, previous reports have not determined whether this apparent propensity of benign ovarian neoplasms to undergo adnexal torsion is due to their greater frequency, as compared with malignant ovarian neoplasms, or to a higher risk of torsion. To address this question, we reviewed our experience with adnexal torsion involving ovarian neoplasms.

Material and methods

The hospital records of 861 patients undergoing laparotomy at Women's Hospital, Los Angeles County–University of Southern California Medical Center from July 1, 1975, to June 30, 1985, with a pathologic diagnosis of ovarian neoplasm were reviewed. These methods have been described previously.[3] Tuboovarian abscesses, nonneoplastic cysts, paratubal cysts, and metastases from other organs were excluded. Calculation of the frequency of various types of ovarian neoplasms was performed with regard to both malignant potential and histologic type. Torsion was defined as a minimum of 360 degrees rotation of the adnexum. Rates and relative risks with 95% confidence intervals were calculated.

Results

Adnexal torsion was identified in 98 patients with an ovarian neoplasm (11%). Ninety-three patients with adnexal torsion had benign ovarian neoplasms (95%), three had neoplasms of low malignant potential (3%), and two had malignant ovarian neoplasms (2%).

The risk of torsion in premenopausal women did not differ from that in postmenopausal women, when controlled for malignancy. Moreover, parity did not appear to affect the risk of an ovarian neoplasm undergoing torsion (data not shown).

Benign ovarian neoplasms had a 12.9-fold increased risk ($p < 0.001$) of being involved in adnexal torsion, as compared with malignant ovarian neoplasms (Table I). Neoplasms of low malignant potential had an 8.7-fold increased risk of torsion as compared with malignant ovarian neoplasms, although this was not statistically significant.

Among the benign ovarian neoplasms that underwent adnexal torsion, no single tumor type appeared to have a predilection for undergoing torsion (data not shown). This was true for neoplasms of low malignant potential and malignant ovarian neoplasms. The rates of torsion were 9.5 per 100 women with neoplasms of epithelial origin, 13.6 for germ cell neoplasms, and 10.5 for stromal neoplasms ($p > 0.05$).

Comment

The clinical impression that malignant ovarian neoplasms are less likely than benign ovarian neoplasms to undergo adnexal torsion has been reported previously.[1, 2] Because malignant ovarian neoplasms are less common than benign ovarian neoplasms, this discrepancy in relative frequency could account for this difference.[3] Alternatively, this low probability of malignant ovarian neoplasms undergoing torsion may be explained by their ability to adhere to local structures. This may be caused by inflammation, adhesions, or local invasion.

From the Department of Obstetrics and Gynecology, Women's Hospital, University of Southern California School of Medicine.
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Reprint requests: Marianne Sommerville, MD, Women's Hospital, Room L-1009, 1240 N. Mission Road, Los Angeles, CA 90033.
6/1/24930

Table I. Rates and relative risks of adnexal torsion by malignant potential of ovarian neoplasm

	No. of patients with ovarian neoplasms undergoing torsion	*Total no. of patients with ovarian neoplasms*	*Rate**	*Relative risk†*	*95% Confidence interval*
Benign	93	650	14.3	12.9	10.2-15.9
Borderline	3	31	9.7	8.7	1.0-24.8
Malignant	2	180	1.1	1.0	Referent
TOTAL	98	861	—	—	—

*Per 100 patients.
†On the basis of an index rate of 1.1 for malignant neoplasms.

Earlier descriptions of ovarian neoplasms have been limited in scope and have not usually provided rates of torsion by tumor type.[2] Although Hibbard[1] did not use statistical tests, he compared rates of torsion by tumor type. Our study confirms that malignant ovarian neoplasms are less likely to undergo adnexal torsion than benign ovarian neoplasms. Whether this is also true for neoplasms of low malignant potential is not clear because of limited numbers of such case.

In summary, ovarian neoplasms that undergo adnexal torsion are usually benign. These findings should be helpful to the surgeon in choosing the operative approach in surgical emergencies and in patients in whom more thorough preoperative evaluation may be impossible.

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Laparoscopic pelvic lymphadenectomy in the staging of early carcinoma of the cervix

Denis Querleu, MD,[a] Eric Leblanc, MD,[b] and Bernard Castelain, MD[c]
Roubaix and Lille, France

Laparoscopic pelvic lymphadenectomy was performed in 39 patients. An incision of the peritoneum between the round and infundibulo-pelvic ligament on each side gave access to the retroperitoneal space. Subsequently, laparoscopic surgery allowed precise dissection of external and internal iliac vessels, umbilical artery, and obturator nerve. The peritoneum was left open, and the lymph was drained into the peritoneal cavity. No lymphocele was observed. Three to 22 (mean, 8.7) nodes were removed, and there was no significant morbidity. Sensitivity and specificity were 100% in this preliminary experience. It is thus possible to remove the first-line regional lymph nodes of the cervix for pathologic examination. Because "skip" metastases are quite rare in early cervical carcinoma, the risk of missing a positive node is low. Brachytherapy alone, vaginal surgery, or, in microinvasive carcinoma, conization alone can be applied safely without the need of a staging laparotomy in cases with negative nodes. (AM J OBSTET GYNECOL 1991;164:579-31.)

Key words: Laparoscopic surgery, carcinoma of the cervix, pelvic lymphadenectomy

The presence of lymph node metastasis is the most significant prognostic factor in carcinoma of the cervix, particularly in those patients with early stage tumors. The management of a patient with invasive carcinoma of the uterine cervix is based on the results of both the clinical examination and diagnostic imaging. Furthermore, pathologic examination identifies those patients with International Federation of Gynecology and Obstetrics (FIGO) stage IA cervical carcinoma in whom there is a risk of lymph-node metastases.

Current clinical staging methods are inaccurate in the detection of metastases to the pelvic lymph nodes. The sensitivity of lymphangiography is less than 30%.[1] This technique does not allow the visualization of internal iliac and other medial node groups. Computed tomographic scanning and magnetic resonance imaging are quite insensitive if the nodes are not macroscopically enlarged.[1-3] Finally, lymphoscintigraphy is too unreliable for routine use.[4]

Lymph node biopsy is thus the only reliable method to appraise the status of pelvic lymph nodes. However, the pathologic specimens are taken either during a presurgical staging laparotomy, which adds significant morbidity, or during the surgical step of treatment, at a time when primary decisions have already been made. Attempts at pelvic lymph node removal by a retroperitoneal endoscopic approach have previously been described.[5,6] Progress in laparoscopic surgery provides the opportunity to remove the primary regional lymph nodes with minimal surgical trauma. We describe in this paper the surgical technique of obturator, external iliac, and hypogastric lymphadenectomy with the use of the laparoscope.

From the Clinique Universitaire de Gynécologie-Obstétrique, Pavillon Paul Gellé, Roubaix,[a] and the Département de Chirurgie[b] and the Département de Radiothérapie,[c] Centre Oscar Lambret, Lille.
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Reprint requests: Denis Querleu, Pavillon Paul Gellé, 91 Avenue Julien Lagache, 59100 Roubaix, France.
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Patients and methods

The surgical technique is essentially the same in every case. No uterine cannulation is necessary, and the patient is placed in the supine position without any flexion of the hips. While the patient is under general anesthesia with tracheal intubation, a pneumoperitoneum is created and an 11 mm laparoscope (Karl Storz, Germany) is inserted through a minimal umbilical incision. A video camera is installed. After observation of the liver surface, the appendix, and the internal genitalia, two 5 mm incisions are made in the right and left inguinal areas, and trocars are inserted into the abdomen. Ancillary 4.5 mm scissors, prehensile forceps, and irrigation-aspiration devices (Micro-France, Bourbon-l'Archambault, France) are consequently available throughout the procedure. An additional opening is made in the midline above the symphysis pubis, and an 8 mm trocar is placed so that the surgeon is ready at any time to use endoscopic clips (Ethicon, Paris) and later to remove lymph nodes from the abdomen with a three-arm retractable Dargent forceps (Lépine, Lyon).

Pelvic lymphadenectomy is performed on each side as follows. External iliac vessels and umbilical artery are identified under the peritoneal surface. The operation begins with an incision of the pelvic peritoneum be-

tween the round and infundibulopelvic ligaments, parallel to the axis of the external iliac vessels. The round ligament is grasped with a forceps, and the peritoneum is cut, then easily torn to open the whole area between the round and infundibulopelvic ligaments. The paracystic space is opened and then widened by blunt dissection between the umbilical artery medially and the external iliac vessels laterally. The pelvic floor is easily reached without any bleeding. The instrument that grasps the round ligament is freed, closed, placed in the paravesical space, and retracts the umbilical artery medially. The cellulolymphatic area below the external iliac vein is then clearly visible and safely dissected. The obturator nerve is identified and dissected caudally to the point at which it leaves the pelvis. The inferior aspect of the external iliac vein is separated by blunt dissection with a closed forceps. The fatty tissue between the obturator nerve and the external iliac vein is then grasped and thoroughly separated from the pelvic wall. At this point, the pelvic bone and the internal obturator muscle are exposed. Care must be taken to avoid injury to retropubic veins and the venous anastomosis between obturator and external iliac veins. However, the magnification that is provided by the laparoscope placed at a short distance from the surgical field allows precise dissection of the vessels, and such an injury has never occurred in our experience. The caudal part of the connective tissue pedicle is then detached from the area of the obturator foramen and of the femoral canal. It is separated by gentle traction. Bleeding is quite unusual at this step, perhaps as a consequence of pressure that is induced by the pneumoperitoneum. The tissue flap is then firmly grasped and moved cranially; it is carefully dissected from the external iliac vein and artery laterally and from the umbilical artery medially. At this point, the cellulolymphatic flap is only attached in the interiliac bifurcation area. It is gently separated by traction and blunt dissection. The tissue sample must then be extracted from the abdomen by means of the Dargent forceps (Lepine). This instrument allows easy removal of the lymph nodes without any risk of contamination of the abdominal wall by tumoral cells, but the specimen may have to be divided into two or three parts. The same technique is carried out on the opposite side. When large adherent pelvic metastases are seen, needle aspiration may replace removal of the node. Hemostasis is then checked. A minimal amount of blood may have to be aspirated. At the end of the procedure, the peritoneum is left open to allow drainage of the lymphatic fluid into the abdomen. In premenopausal patients with mobile ovaries, when brachytherapy is planned, a laparoscopic transposition of the ovaries to the abdominal wall may be performed to avoid ovarian irradiation.

Thirty-nine patients who presented with FIGO stages IB (n = 9), IIA (n = 7), and proximal IIB (n = 13) carcinoma of the cervix underwent pretreatment laparoscopic staging between December 1988 and December 1989. Selection of patients was based on the potential risks of prolonged pneumoperitoneum and the absence of cytologically positive nodes that were visible on computed tomographic scans. Their age range was 28 to 69 years, including 15 patients older than age 50. The pathologic types of the cervical tumors were squamous carcinoma (n = 35) or adenocarcinoma (n = 4). The primary regional lymph nodes were histologically examined by frozen sections and/or pathologically examined in all but two cases. In these last two cases, metastatic epithelial cells were demonstrated on needle aspirates of unresectable nodes.

Five patients with metastatic nodes were given the option of radiation therapy alone. All other patients were operated on later (the same day or 1 to 6 weeks later). Two patients underwent Schauta-Amreich radical vaginal hysterectomy. The last 32 patients had Wertheim abdominal radical hysterectomy with pathologic control of occasional remaining primary and of palpable secondary primitive iliac or paraaortic lymph nodes.

Results

One to 15 nodes were removed from each side (mean, 4.2 on the right and 4.9 on the left side). As a whole, 3 to 22 nodes were available for pathologic examination (mean, 8.7). No unexpected metastatic node was observed at laparotomy in 32 cases. Finally, sensitivity and specificity were 100% in this series.

The procedure typically lasted 90 minutes. Two intraoperative complications were observed. One patient did not tolerate the prolonged pneumoperitoneum, and we had to abandon the procedure after removal of only three nodes. In another case, bleeding from the umbilical artery necessitated bipolar coagulation of the vessel. No unwanted laparotomy was necessary. Clips were never used, and minimal bleedings occurred under irrigation with warm saline solution. The postoperative period was uneventful, and patients were discharged from the surgical ward on the following day in all but one case. In this case, a spontaneously resolutive pelvic hematoma was observed 5 days after laparoscopic pelvic lymphadenectomy and radium application. No lymphocele was observed. No bowel adhesions were noticed at further laparotomy, and spontaneous healing of the peritoneum always occurred without intraperitoneal sequelae. However, dense fibrosis of the retroperitoneal tissue was noticed in some cases.

Comment

Pathologic examination is the gold standard in the detection of lymph node metastasis. Pretreatment surgical staging provides the most sensitive means of di-

agnosing node involvement. However, the cost and risks of open-field pretherapeutic surgical staging preclude its general application in clinical practice, particularly in early stage carcinoma of the cervix, in which the prevalence of node metastasis is rather low. Progress in endoscopic surgery should dramatically reduce the cost of pathologic staging of pelvic nodes.

However, laparoscopy, as well as surgical staging, is useless if suspicious lymph nodes shown by computed tomographic scanning have been proved positive by percutaneous fine-needle aspiration. Furthermore, it is at this time impossible to explore paraaortic nodes by laparoscopy. A retroperitoneal approach is preferable in the paraaortic area.[5] It could be used in patients at risk of secondary lymph node metastasis, that is, in patients with locally advanced tumors or early stage cervical cancers with positive pelvic nodes. However, the poor outcome of patients with paraaortic nodal metastasis, regardless of treatment, does not seem to justify the risks of paraaortic staging for all patients in a noninvestigational setting.

The accuracy of a negative laparoscopic lymphadenectomy is excellent. Sensitivity is 100% in this series. The risk of "skip" metastases (primitive iliac or paraaortic nodes without pelvic node involvement) is very low in cases of early stage invasive cervical cancer (<2%)[6] and seems to occur only in patients with large tumoral volume.

As a consequence, FIGO stage I, IIA, or proximal IIB cases with negative pathologic staging may be cured by vaginal surgery alone or by brachytherapy alone without the need for external radiotherapy. On the other hand, radical hysterectomy does not seem necessary when metastatic nodes are present.[7] The consequent reduction of risks and cost justifies the additional general anesthesia and the short hospital stay of a diagnostic laparoscopy. At this time, our treatment protocol for early stage "operable" cervical carcinoma includes laparoscopic lymphadenectomy. Patients with positive nodes are given the option of radiotherapy alone. Patients with negative nodes have had brachytherapy and then abdominal surgery during the first step of the investigation; since the accuracy of staging laparoscopy has been proved, we suggest only radical vaginal surgery or brachytherapy.

Some FIGO stage IA2 cervical carcinomas without pelvic node metastasis can be treated by cervical conization alone. When hysterectomy is not necessary for the local control of such tumors, laparoscopic lymphadenectomy helps to identify women of reproductive age with microinvasive carcinomas who may benefit from conservative therapy. The rare cases with node metastasis, which justify a radical treatment protocol, can be differentiated from cases with negative nodes in which cervical conization with preservation of fertility is an acceptable treatment.

Laparoscopic lymphadenectomy is a feasible alternative to laparotomy for lymph node staging of carcinoma of the cervix. It helps to reduce the cost, risks, and effects on fertility in the majority of cases of early stage cervical carcinoma. Its risks seem to be low, since no serious surgical complication has been observed in this series. In particular, the absence of lymphoceles is a consequence of the intraperitoneal lymph drainage during the postoperative period before spontaneous peritoneal healing has occurred. However, it should be stressed that prolonged pneumoperitoneum may be contraindicated in patients with cardiac or pulmonary diseases or in obese patients. Moreover, this technique should be used only by surgeons who work in referral centers and who have considerable expertise in gynecologic-oncologic surgery and in surgical laparoscopy.

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Umbilical cord ulceration and intestinal atresia: A new association?

Robert W. Bendon, MD[a], R. Wes Tyson, MD,[b, c] Virginia J. Baldwin, MD,[b, c]
Kathryn A. Cashner, MD,[d] Francis Mimouni, MD,[e] and Menachem Miodovnik, MD[d]
Cincinnati, Ohio, and Vancouver, British Columbia, Canada

In three fetuses, congenital intestinal atresia was associated with linear ulcerations of the umbilical cord. In two cases, hemorrhage was seen from the cord ulcer. Both fetuses required emergency cesarean section for fetal distress and were born anemic. The third fetus was mildly hydropic, attributed to hemorrhage, and was stillborn. The mechanism of the association could not be determined. These cases suggest a risk of prenatal umbilical cord hemorrhage in infants with intestinal atresia. (Am J Obstet Gynecol 1991;164: 582-6.)

Key words: Intestinal atresia, umbilical cord, fetal hemorrhage, ulceration

Massive hemorrhage from the umbilical cord has been reported to occur with some rare lesions such as hemangiomas.[1] Bleeding from ulceration of the cord has seldom been reported.[2] We report three cases with suspected in utero hemorrhage from umbilical cord ulcerations. All three fetuses also had congenital intestinal atresia.

Clinical histories

Case 1. The mother was a 19-year-old, gravida 3 woman with two previous term pregnancies. She smoked cigarettes and took aminophylline for the treatment of asthma.

At 31 weeks' gestation by dates, she was seen in preterm labor with advanced dilatation. Examination suggested polyhydramnios (41 cm fundal height), which was confirmed by ultrasonographic examination.

Artificial rupture of membranes released copious bloody amniotic fluid. Late fetal heart rate (FHR) decelerations developed, followed by an abrupt fall to 50 beats/min. A 1830 gm male infant was delivered by emergency cesarean section. Apgar scores were 1, 3, and 7 at 1, 5, and 10 minutes, respectively. The operative note reported "many small black tags (clots)" along the length of the umbilical cord.

The infant was pale at birth and his initial hematocrit value was 28%. A transfusion of 20 ml of packed red blood cells per kilogram body weight raised the hematocrit value to 53%. The infant was appropriate for gestational age of 31 weeks. A chest radiograph showed mild respiratory distress syndrome and the "double-bubble" sign of duodenal atresia. At 6 days of age, he underwent duodenostomy and gastrostomy for an atresia in the second part of the duodenum. He did well postoperatively and was discharged 40 days later.

He was treated for asthma as an infant. At 4 years of age, he still has asthma and persistent vomiting.

Case 2. The mother was a 27-year-old gravida 2 woman with a previous uncomplicated term pregnancy. Her medical and social history were unremarkable. She was seen at 34 weeks' gestation because of spontaneous rupture of membranes. An ultrasonographic examination after rupture of membranes showed a normal volume of amniotic fluid (suggesting previous polyhydramnios), an immature placenta, and a fetus with aortic stenosis and bowel atresia. The baseline FHR was 100 to 110 beats/min with normal variability on an external monitor.

Four days later, an amniocentesis was performed for maturity studies. The fluid was bloody and unanalyzable. The FHR was monitored and remained reactive with a slow baseline. Eleven hours later a fetal bradycardia in the 60s was sustained for 9 minutes. An emergency cesarean section was performed. A 2020 gm male infant was delivered with Apgar scores of 3 at 1 minute, 4 at 5 minutes, and 6 at 10 minutes. The cord blood had a pH of 7.24 and a base deficit of 6.6. The amniotic fluid was bloody.

The infant was appropriate for a gestational age of 34 weeks, and the birth weight was 2020 gm (70th percentile). Heart rate was 98, respiratory rate was 50, and blood pressure was undetectable. The precordium was hyperactive. The pulses and capillary refill were decreased. An umbilical cord clamp had been placed in

From the Department of Pathology and Laboratory Medicine, University of Cincinnati,[a] Department of Pathology, British Columbia's Children's Hospital,[b] University of British Columbia,[c] Department of Obstetrics and Gynecology, University of Cincinnati,[d] and Department of Pediatrics: Neonatology, University of Cincinnati.[e]
Received for publication June 17, 1990; revised August 16, 1990; accepted August 31, 1990.
Reprint requests: Robert W. Bendon, MD, Department of Pathology, University of Cincinnati Medical Center, 231 Bethesda Ave., Cincinnati, OH 45267-0529.
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the delivery room approximately 2 cm above the abdomen. A 4 to 5 cm stump of umbilical cord remained above the clamp. This portion showed a 2 cm linear ulcer, 2 to 4 mm wide, that exposed one umbilical artery. The vessel appeared necrotic with an adherent blood clot. The hematocrit value was below detection. The infant was resuscitated with 70 ml of packed red blood cells and 20 ml of plasmanate human plasma protein fraction with intravenous dopamine at 10 μg/kg per minute. At 30 minutes after birth his blood pressure was 36/16 with a mean arterial pressure of 29.

On day 9 after birth, he underwent exploratory laparotomy, at which time multiple atretic areas of the distal jejunum were found and resected. Echocardiography showed mild to moderate tricuspid insufficiency, enlarged right ventricle, small perimembranous ventricular septal defect, a borderline small aortic root, and abnormal motion of the posterior mitral leaflet. The infant was discharged from the Neonatal Intensive Care Unit and is followed up by cardiologists.

Case 3. The mother was a 21-year-old primigravid woman. Her pregnancy was complicated in the first trimester by bronchitis treated with penicillin, and in the last month by a 15-pound weight gain and increasing abdominal girth. She smoked 10 to 15 cigarettes per day.

At an estimated gestation of 30 weeks, she was admitted to the hospital in preterm labor, but labor did not progress. On the last evening, a FHR tracing showed a rate of 170 to 180 beats/min. Four hours later, a heart beat could not be detected and an ultrasonographic examination confirmed fetal death. Artificial rupture of membranes produced clear fluid for 15 minutes and then bloody fluid and a 3 to 4 cm blood clot. A 1700 gm, stillborn female fetus was delivered vaginally. Autopsy revealed development consistent with 30 weeks' gestation, preampullary duodenal atresia, and linear umbilical cord ulcerations.

Pathologic findings

Case 1. The fixed placenta was normal in weight, dimension, and gross examination. Ten centimeters of umbilical cord were attached and another 7 cm were received separately. The cord was stained brown. Gross ulcerations with small adherent clots followed the spiral of one dilated umbilical artery (Fig. 1, *A*). Sections of the placenta, umbilical cord, and roll of fetal membranes were processed for histologic examination. Stains performed on the umbilical cord included hematoxylin-eosin, Prussian blue, alcian blue–PAS, Grocott-Gomori methenamine-silver nitrate, Giemsa, Gram, acid fast, elastin, and immunoperoxidase localization of cytokeratin.[3]

Histologically, the umbilical ulcer bed was filled with amorphous debris, red blood cells, squames, and plump, often multinucleate or clumped, epithelial cells (cytokeratin-positive) (Fig. 2, *A*). The ulcer bed was superficial to and between the arteries. No organisms were detected. There was acute inflammation of the umbilical vein but not of the arteries. Placental findings also included mild acute inflammation of the intervillous, subchorionic fibrin, mildly hydropic villi, and Prussian blue–negative, pigment laden macrophages in the amnion and chorion.

Case 2. The drained, trimmed weight of the fresh placenta was 380 gm. The umbilical cord was 22 cm long and inserted centrally. The cord had a tight spiral with a depression along the inner groove showing focal ulceration (Fig. 1, *B*). The fetal membranes had a light grey-green discoloration. The serial cut surfaces of the placenta were pale. Sections of the umbilical cord, membranes, and placenta were studied histologically and stained with hematoxylin-eosin and Prussian blue for iron.

Microscopically, the ulcer ran between the two umbilical arteries. The ulcer had the same histologic characteristics as case 1 (Fig. 2, *B*). One artery was markedly dilated, but not necrotic. Acute inflammation was not evident in either the umbilical cord or the membranes. There were plentiful pigment-filled macrophages in the fetal membranes. The pigment did not stain for iron. The placental villi were mildly hydropic.

Case 3. The trimmed placenta weighed 375 gm and appeared meconium-stained. The three-vessel umbilical cord was received in two pieces, 16 and 25 cm long, respectively. There were two linear ulcers following the course of the umbilical vessels (Fig. 1, *C*). The cord had a brown discoloration. The maternal surface of the placenta had an ill-defined area of compression and a 10 cm adherent marginal thrombus. The cut sections of the placenta were pale.

Microscopically, the ulcer bed had the same histologic characteristics as cases 1 and 2, but in addition focally overlaid the vein (Fig. 2, *C*). There was mild acute vasculitis of the arteries and vein without necrosis of the vessel wall. The fetal membranes contained rare neutrophils and meconium pigment contained within macrophages. There was focal, mild nonspecific villitis.

At autopsy the 1700 gm female fetus was estimated to be 32 weeks' gestation with early maceration. There was mild hydrops with periorbital edema, 10 ml of fluid in the pleural spaces, 2.5 ml pericardial effusion, and 3 ml of ascites. There was a preampullary membranous duodenal atresia with a moderately dilated stomach. Evidence of acute fetal distress included intrathoracic petechiae and sufficient amniotic debris to suggest aspiration. Chromosome analysis revealed a normal 46,XY karyotype.

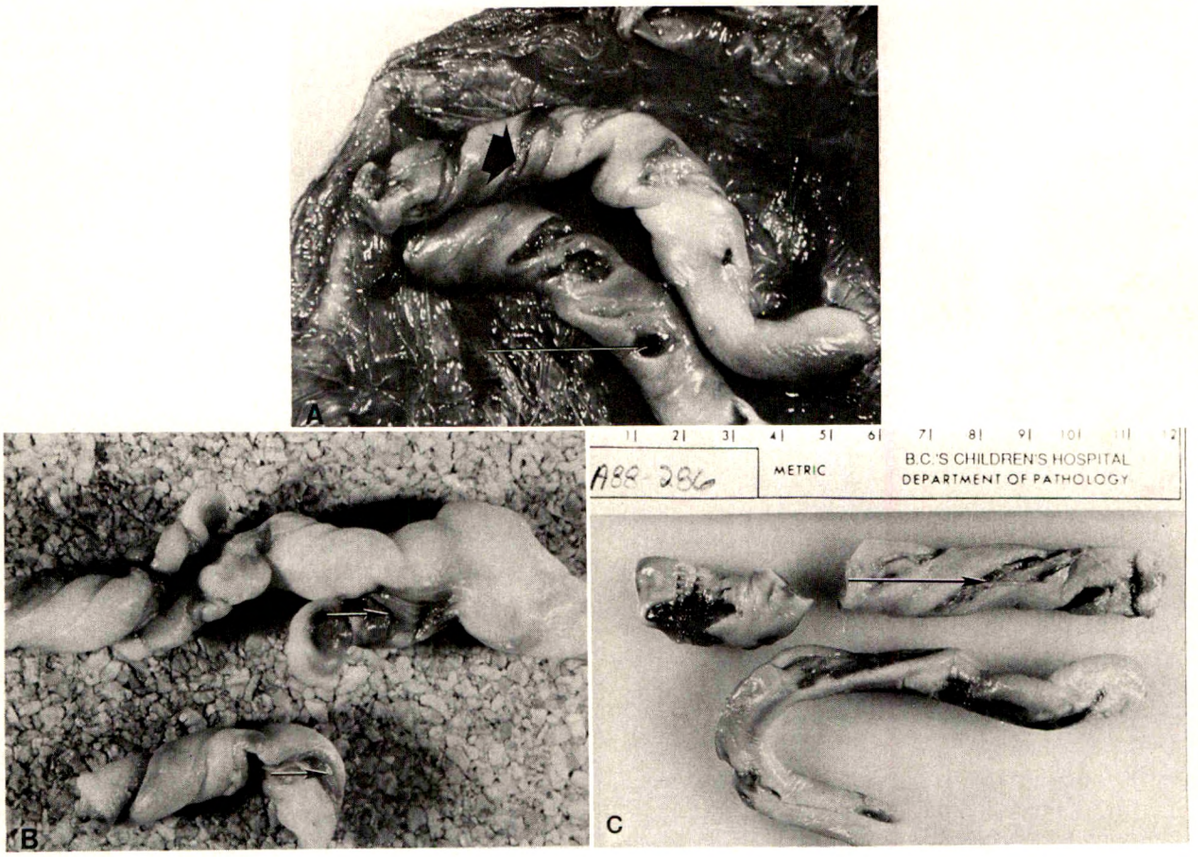


Fig. 1, A. Case 1, two segments of umbilical cord lie on placenta. *Arrow* points to adherent clot. *Arrowhead* points to exposed umbilical cord artery. **B,** Case 2, two segments of umbilical cord lie on corkboard. *Arrows* point to ulcers in deep spiral groove of cord. **C,** Case 3, three segments of umbilical cord show spiral ulcers *(arrow)*.

Comment

These three cases had similar pathologic conditions (an ulceration of the umbilical cord), congenital intestinal atresia, and probable in utero hemorrhage. Three tentative conclusions can be drawn from the case findings. First, the pathologic findings provide constraints on the possible mechanism of ulcer formation. Second, clinically severe hemorrhage from the ulcers occurred in utero in at least two cases. Third, the ulcers were associated with fetal intestinal atresia but no mechanism can be determined, as yet, from the clinical or pathologic observations.

The three cases shared the following anatomic findings. Grossly, the ulcers paralleled the vascular helix. They were superficial to the arteries and in one case to the vein. The ulcer beds contained necrotic debris but little inflammation. No organisms were found with special stains; no viral inclusions were identified. Large, sometimes multinucleated, cells in the ulcer bed were epithelial.

The ulcers could have been linear for one of two reasons. Possibly, the epithelial surface was more susceptible to injury in a linear pattern related to the underlying vessels because of varying oxygenation or the effects of mechanical pulsation. Alternatively, an injurious substance dissected along channels of least resistance in the nonhomogeneous Wharton's jelly with epithelial ulceration occurring as a result. However, the initiating injury or injurious substance remains undetermined.

The hemorrhages into the amnionic cavity appear to have been from the exposed umbilical vessels. In the first case, adherent clotted blood was seen on the umbilical cord at cesarean section by the obstetrician. Necrosis of the umbilical artery was confirmed microscopically. In the second case, hemorrhage from the attached cord segment was seen by the neonatologist. Both infants were anemic at birth. The third fetus was hydropic, which can occur after fetal hemorrhage.[4] No other cause of hydrops was detected. Fetal hemorrhage could have occurred from the umbilical cord ulcer, although no specific site was found.

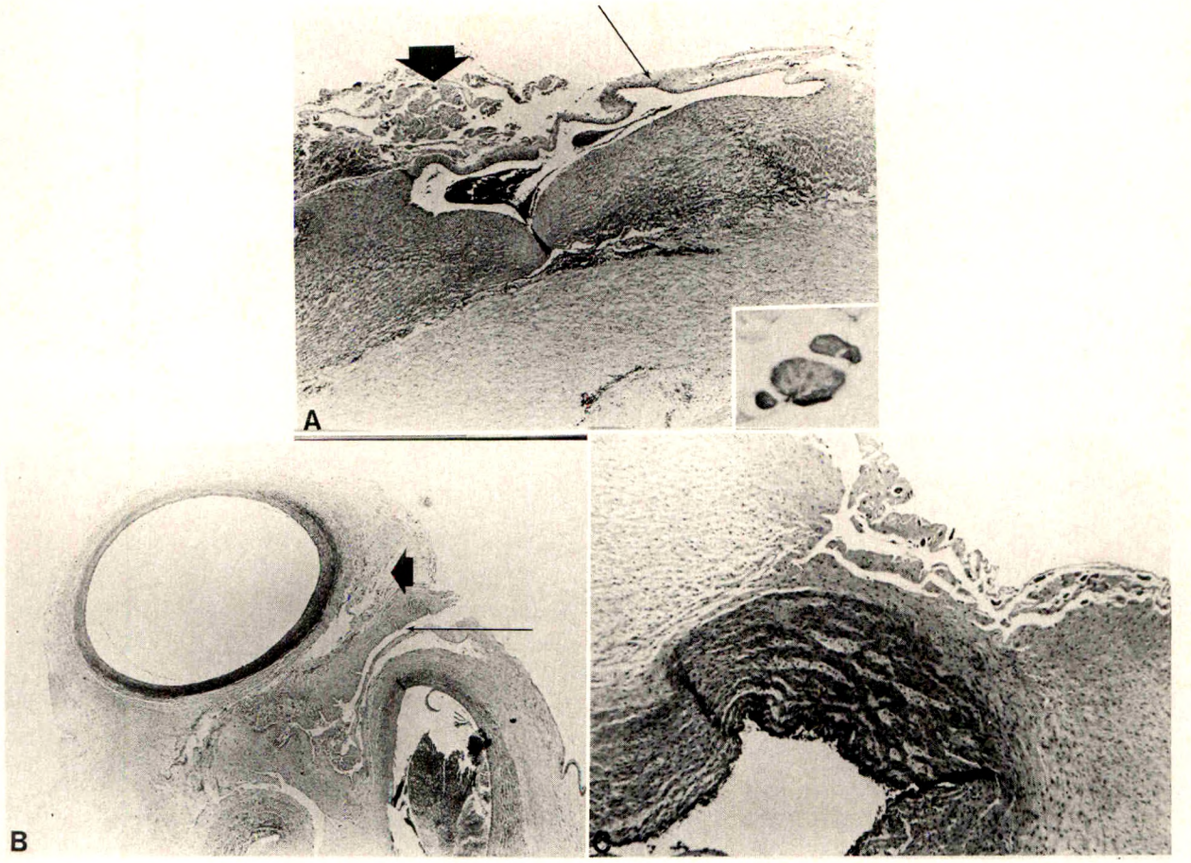


Fig. 2, A. Case 1, *arrow* points to thinned, superficial wall of umbilical artery that has been exposed by ulcer. *Arrowhead* points to ulcer bed debris with large epithelial cells. (Hematoxylin-eosin stain; ×33.) *Inset*, Anticytokeratin positivity of large epithelial cells. (Hematoxylin counterstain; ×250.) **B,** Case 2, ulcer bed *(arrowhead)* is alongside deep, nonpathologic, groove between arteries *(arrow)*. (Hematoxylin-eosin stain; ×25.) **C,** Case 3, ulcer overlies umbilical vein, which also shows some acute inflammation of vessel wall. Ulcer bed shows large epithelial cells. (Hematoxylin-eosin stain; ×12.5.)

We considered three general hypotheses to account for the association of umbilical cord ulceration with intestinal atresia: vascular reactivity, gastric reflux, and epithelial abnormalities. In the first hypothesis, an abnormal vascular reactivity could produce both lesions. Experimental and clinical evidence suport an ischemic origin of congenital intestinal atresia,[5] and the umbilical cord arteries have the capacity to maintain a prolonged vascular spasm after delivery.[6] If such a spasm was initiated in one artery in utero it might cause an ischemic ulcer of the umbilical cord. In two cases, the mothers smoked cigarettes, a potential source of vasoconstrictor; one of these mothers and her infant have asthma, possible evidence of abnormal smooth muscle reactivity. In the second hypothesis, the intestinal atresia might produce reflux of gastric or duodenal contents that could initiate an ulcer. The hemosiderin-negative, green-brown pigment in the amniotic and chorionic macrophages may reflect phagocytosis of bilious reflux. This hypothesis is supported by the observation of increased optical density of the amnion fluid at 450 nm in a case of duodenal atresia.[7] In the third hypothesis, a primary epithelial abnormality might produce both intestinal atresia and the cord ulcer similar to the association of epidermolysis bullosa with intestinal atresia.[8] However, our findings could not prove or disprove any of these hypotheses.

Review of the English literature found no identical cases. Cords with absence of Wharton's jelly have a superficial resemblance to our umbilical cord ulcers, but are pathologically and clinically different.[9,10] A morphologically very similar ulcer that produced lethal prenatal hemorrhage was reported in association with an omphalomesenteric duct extending from the ileum.[2] Within the umbilical cord, the duct was lined with gastric mucosa, analogous to some Meckel's diverticula.

Microscopy showed that the duct was the source of the ulcer. Another related case with severe in utero hemorrhage and distal ileal atresia was attributed to an umbilical cord hemangioma.[11]

The association of congenital intestinal atresia and umbilical cord ulceration and hemorrhage may be clinically important. The delay in recognition of the potential for hemorrhage probably contributed to the morbidity and mortality in our cases. If the risk of hemorrhage is recognized in prenatally diagnosed congenital bowel atresia, this may permit delivery before severe blood loss has occurred. Although present in only two of our three cases, a short umbilical cord may be an additional indicator of risk. In two of our cases, the umbilical ulcer was not recognized clinically even after delivery. This suggests that the lesion may occur more frequently than the literature review would indicate.

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Mechanisms of hemolysis and anemia associated with acute antepartum pyelonephritis

Susan M. Cox, MD, Paul Shelburne, MD, Ruble Mason, MT, ASCP, Stephen Guss, MS, and F. Gary Cunningham, MD

Dallas, Texas

Anemia develops in about a fourth of women whose pregnancy is complicated by pyelonephritis, although its exact mechanism has not been defined clearly. In this study of 18 women with antepartum pyelonephritis, although only a third had anemia (hematocrit <30 vol/dl), there was evidence for hemolysis in all 18. Specifically there was a mean decrease in hematocrit of 5 vol/dl from admission to discharge. With scanning electron microscopy, we compared erythrocyte morphologic aberrations that were found in women with renal infection with those of normally pregnant women, and the former had significantly increased proportions of echinocytes in particular, but schistocytes and spherocytes were increased also (total 10.3% vs 1.4%, $p < 0.0001$). These changes, especially echinocytosis, have been induced in vitro by lipopolysaccharide, and they are known to lead to premature red blood cell destruction in vivo. We conclude that hemolysis with subsequent anemia in pregnant women with pyelonephritis is caused by lipopolysaccharide-induced red blood cell membrane damage. (Am J Obstet Gynecol 1991;164:587-90.)

Key words: Pyelonephritis, anemia, hemolysis

As many as 10% to 20% of women with antepartum pyelonephritis have culture-proved bacteremia, which is usually caused by *Enterobacteriaceae*.[1, 2] Whereas overt sustained hypotension is rare, other presumed endotoxin-mediated effects are more common. For example, with severe renal infection during pregnancy, endotoxemia has been implicated as the cause of transient renal dysfunction and pulmonary injury.[3, 4] In these and other studies, it was also found that anemia develops in about a fourth of women with pyelonephritis.[5, 6] Although the exact mechanism(s) by which anemia develops is unclear, it is presumed that hemolysis is caused in some way by endotoxin-mediated erythrocyte membrane injury.[7] In this investigation we characterized erythrocyte morphologic changes that are related to acute anemia in pregnant women with pyelonephritis.

Patients and methods

We studied 18 pregnant women who were admitted to Parkland Memorial Hospital for treatment of pyelonephritis. Ten of these women had attended a prenatal clinic, and the other eight had received no prenatal care. All presented with flank pain, fever, chills, nausea and vomiting, and tenderness to fist percussion at one or both costovertebral angles. Renal infection was confirmed by culture of catheterized urine and *Escherichia coli* was recovered from 13, *Klebsiella pneumoniae* from three, and *Enterobacter* organisms from two. Five of the 18 women had positive blood cultures, three for *E. coli* and two for *Enterobacter* organisms. Along with vigorous hydration, 14 women were treated with a β-lactam agent and aminoglycoside antimicrobial combined, and the other four were treated with a β-lactam agent alone. All women were given intravenous antimicrobial treatment until they were afebrile for at least 24 hours.

In addition to blood sampling as dictated by clinical care, blood was obtained for scanning electron microscopy studies. Blood for hematologic studies was also obtained from 25 women with normal pregnancy who were admitted at term in early labor.[8] None of these patients or control subjects had evidence for a chronic hemolytic process or other erythrocyte disorder, including sickle cell trait. Peripheral blood was collected into vacuum tubes that contained ethylenediaminetetraacetate and was processed within 1 hour. One milliliter of blood was centrifuged at 1000 *g* for 3 minutes. The plasma was discarded and red blood cells were suspended in a fixative solution of 1.25% glutaraldehyde in cacodylate buffer (0.1 mol/L). Three drops of freshly anticoagulated blood was added to the fixative and mixed gently. After it had settled at room temperature for 1 to 3 hours, the suspension was spun for 4 minutes at 1000 *g*, the supernatant was dis-

From the Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center.
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Reprint requests: Susan M. Cox, MD, Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9032.
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Table I. Mean hematocrits in 18 women with antepartum pyelonephritis

Patient group	Hematocrit		Day 1†	Discharge‡	Delivery
	Clinic*	Admission			
Prenatal clinic (n = 10)					
Mean	37.2	34.1	28.5	29.2	35
Range	29-40	24-36	23-35	25-35	33-39
Nonclinic (n = 8)					
Mean	—	31	26.1	27.5	33
Range		25-38	23-31	23-34	27-40

*Clinic hematocrits determined 1 to 12 weeks before admission.
†Day 1 hematocrits performed 8 to 12 hours after admission.
‡p = 0.0008 when compared with admission hematocrits.

carded, and the cells were resuspended gently in 2 ml of 0.1 mol/L cacodylate buffer. The centrifugation-resuspension step was repeated twice, and the last suspension was refrigerated at 4° C until the cell membranes were prepared for microscopic examination.

From each subject and her control, 500 erythrocytes from contiguous fields were examined with a JEOL model JSM-35 (JEOL USA, Peabody, Mass.) scanning electron microscope. Cells were categorized according to the classification of Bessis[9] as normal discocytes or as abnormal echinocytes, schistocytes, or spherocytes. The proportion of abnormal erythrocytes was determined for each woman. Differences in the mean proportions of abnormal red blood cells among the groups of study subjects and the respective control subjects were tested by means of the Mann-Whitney test for statistical significance.[10] This nonparametric test was used because fractional values do not generally satisfy the normal assumptions that are required for parametric testing.

Results

The demographic characteristics of the 18 women with antepartum pyelonephritis were not different from those of the general obstetric population at Parkland Hospital. In 11 of the 18 women, the hematocrit decreased 5 vol/dl or more from admission to discharge (mean 4 days). In the remaining seven women, the mean decline was 3 vol/dl. Shown in Table I are the mean hematocrits that were determined in clinic, at admission, after hydration, and at discharge. In both clinic and nonclinic groups, the hematocrit decreased 4 to 5 vol/dl during hospitalization. Evidence that this was not simply due to rehydration is illustrated by the 10 women who had prenatal care. These women had taken iron supplements and had not been previously anemic. In spite of this, these women had a mean decrease in hematocrit of 8 vol/dl when determinations made during hospitalization were compared with the most recently obtained hematocrit from the clinic. Moreover, the posthydration hematocrit was decreased by a mean of 9.1 vol/dl (range 4.4 to 14 vol/dl) compared with the last prenatal hematocrit. By the time of delivery the mean hematocrit in both clinic and nonclinic women exceeded 30 vol/dl, and it was <30 vol/dl in only two nonclinic patients.

None of these women who were tested had a positive Coombs' test. Although the serum bilirubin level was not appreciably elevated (mean 0.7 mg/dl; range 0.4 to 1.7 mg/dl), urine urobilinogen was usually detected. Modest reticulocytosis in spite of severe infection provides further evidence for hemolysis. The mean reticulocyte count on admission was 1.8% (range 0.2% to 3.0%).

In Table II the mean proportions of abnormal erythrocytes from women with pyelonephritis are compared with those from normally pregnant control women. A significantly increased proportion of abnormal erythrocytes was found in women with pyelonephritis (10.3% vs 1.4%, $p < 0.001$). The most common morphologic abnormality was echinocytosis, but the proportions of schistocytes and spherocytes were also increased significantly. Representative erthrocyte abnormalities from one of these women are shown in Fig. 1.

There was no apparent correlation between the magnitude of hemolysis and abnormal red blood cell forms. By way of examples at the extremes, in one woman with 36% abnormal erythrocytes there was a 5 vol/dl fall in hematocrit; in another with 2.8% abnormal forms there was a 4 vol/dl decrease. In 15 women serial observations were performed during hospitalization to search for a trend over time. In seven women the percentage of abnormal cells decreased, in six it increased, and in two (who had 2.7% and 1.4% abnormal forms) there was no change.

Comment

Acute pyelonephritis during pregnancy causes multiple organ system derangements that apparently are mediated by bacterial endotoxin (lipopolysaccha-

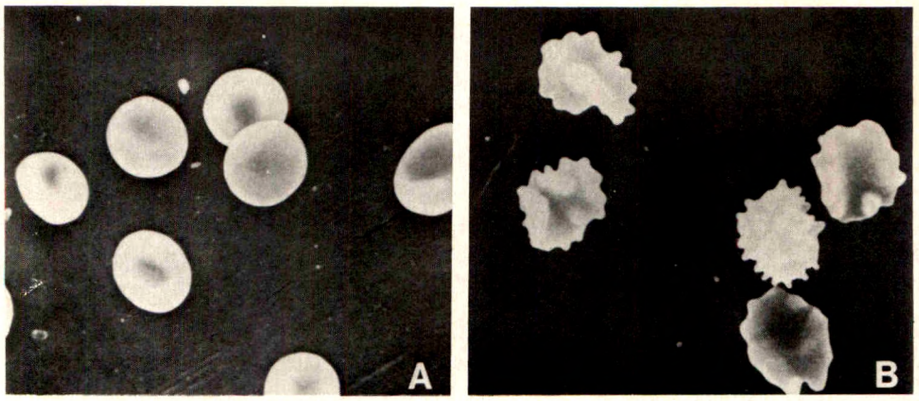


Fig. 1. A, Scanning electron microscopy of peripheral blood taken from a woman at the time of admission for acute antepartum pyelonephritis. Normal discocytes shown constituted 97% of the erythrocytes. **B,** Peripheral blood from the same woman 24 hours later and showing echinocytes that constituted 7% of erythrocytes (schistocytes were noted in 6%).

Table II. Mean proportion of normal and abnormal erythrocytes in women with pyelonephritis compared with normally pregnant control women

	Erythrocytes (%)				
		Abnormal			
Patient group	*Normal discocytes*	*Total**	*Echinocytes**	*Schistocytes**	*Spherocytes†*
Pyelonephritis (n = 18)					
Mean	89.7	10.3	7.5	2.2	0.6
Range		1.4-36	1.4-33	0.6-5.4	0.2-2.4
Control (n = 25)‡					
Mean	98.6	1.4	0.5	0.5	0.4
Range		0.4-4.6	0-1.2	0-2.8	0-1.2

*$p < 0.0001$, pyelonephritis versus control.
†$p = 0.03$, pyelonephritis versus control.
‡From Cunningham et al.[8]

ride). These include, but are not limited to, hypothalamic, hepatic, renal, and pulmonary aberrations.[2-4] Interleukin-1 and other cytokines are released by activated macrophages and indirectly affect the hypothalamus to cause chills and fever.[11] Clinical evidence for kidney damage has been reported in nonpregnant human beings with severe sepsis,[12, 13] and Whalley et al.[3] described reversible renal dysfunction in pregnant women with pyelonephritis. Also in pregnant women with renal infection, Cunningham et al.[4] described pulmonary injury, which is characterized by adult respiratory distress syndrome in its worst form, and attributed this to endotoxemia. Thrombocytopenia commonly accompanies septicemia, and Kelton et al.[14] reported that this may be caused by endotoxin-induced platelet-associated immunoglobulin. Although poorly characterized, acute hemolysis accompanied by anemia may develop in persons with *E. coli* septicemia.[7, 15]

It was previously reported that anemia, defined by a hematocrit <30 vol/dl, develops in 25% to 30% of women who are hospitalized for antepartum pyelonephritis.[5, 6] The incidence of anemia in the current investigation was similar, and although only a third of the women had anemia, there was evidence for hemolysis in all, which was manifested by an average decrease in hematocrit of approximately 5 vol/dl. Significantly, although none of the 10 women with presumably sufficient iron who attended clinic had been anemic previously, eight had anemia that persisted at discharge. Almost all of these women with pyelonephritis had substantively increased erythrocyte morphologic abnormalities that were identified by scanning electron microscopy. Specifically, there were significantly increased numbers of echinocytes and schistocytes, and, to a lesser extent, spherocytes when red blood cells from women with renal infection were compared with those from normally pregnant women. Although we did not conduct studies to determine the longevity of these morphologic aberrations, the mean hematocrit for clinic and nonclinic patients increased

by 5 vol/dl from discharge to delivery, and only two of the 18 women were still anemic by the time of delivery.

It seems probable that hemolysis with subsequent anemia is caused by lipopolysaccharide-induced red blood cell membrane damage. It has been shown previously that a number of in vivo extraerythrocytic pertubations induce predominantly echinocytosis.[8] Similar erythrocyte membrane alterations were produced in vitro by Warren et al.[16] who described a concentration-dependent endotoxin effect on red blood cell membrane curvature. They also described a reversible discocyte-to-echinocyte transformation in erythrocytes that were treated with endotoxin. Previously, Cooper et al.[17] reported that shortened erythrocyte survival in echinocytosis resulted from "conditioning," which describes progressive membrane loss after splenic passage. Moreover, at least in bacteremic animals, endotoxin is released when antimicrobial therapy is given,[18] and we did not detect maximal erythrocyte aberrations typically for at least 24 hours after therapy was begun. Because of these findings, it seems reasonable to conclude that endotoxin-induced erythrocyte membrane changes lead to acute hemolysis and cause anemia that may accompany antepartum pyelonephritis. In this scheme red blood cells that are rendered echinocytic cannot readily traverse the splenic slits, and as membrane loss progresses, echinocytes are converted to schistocytes or spherocytes that are then rapidly destroyed.

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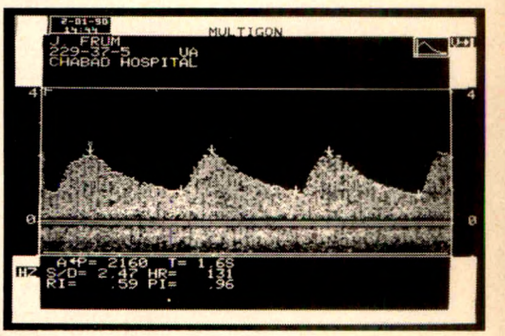
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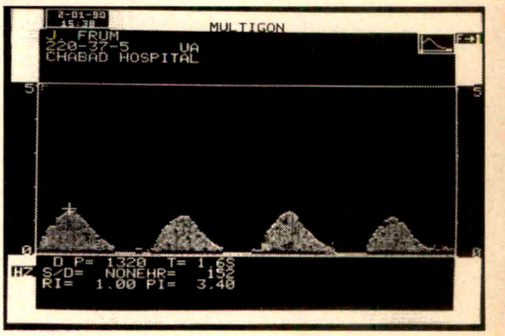


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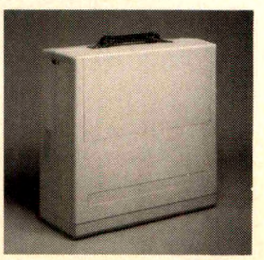
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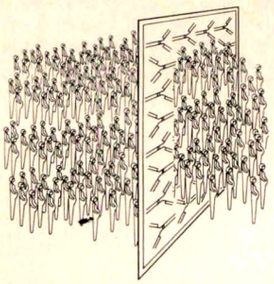
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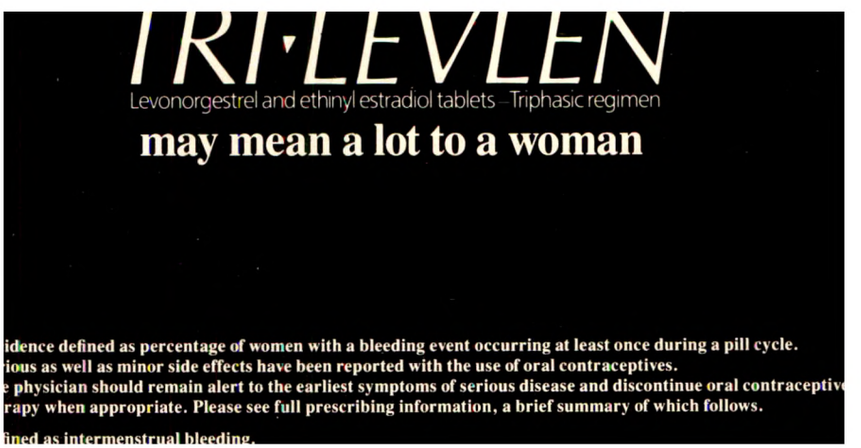
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CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.
MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.
RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.
ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.
PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.
2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.
3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.
4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.
5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It i recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If th patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first misse period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should b apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light o these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form o contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precis information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estroge combinations to induce withdrawal bleeding should not be used as a test of pregnancy.
6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. I one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, a increased risk was apparent between 6 and 12 months of use.
7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage o patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase i triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipi metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of thes findings remains to be defined.
8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertensio may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in user and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated wit the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely t develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to norma after discontinuing the drug.
9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent o severe, requires discontinuation of OCs and evaluation of the cause.
10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. I breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. I undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule ou pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing t an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea o young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation o OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.
11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.
12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity an quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mother receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should b deferred until the infant has been weaned.
Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatmen and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 yea without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4 OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac o renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

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Information for the Patient—See Patient Package Labeling
Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.
Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.
Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."
Nursing Mothers—See "Warnings."
Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.
There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.
The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted.: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.
Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.
Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.
For full details on dosage and administration see prescribing information in package insert. 60611-1 5/86

Reference: 1. Data on file, Berlex Laboratories, Inc.

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Fetal panting: Yet another response to the external vibratory acoustic stimulation test

David M. Sherer, MD, Jacques S. Abramowicz, MD, Mary Lou D'Amico, RN, Tamara Allen, BS, RDMS, and James R. Woods, Jr., MD

Rochester, New York

Fetal panting at a rate of 119 respiratory movements per minute, persisting for 40 minutes after external vibratory acoustic stimulation, was noted in a fetus at 33 weeks' gestation. Subsequent fetal outcome was good. The possible underlying pathophysiologic mechanism is discussed. (AM J OBSTET GYNECOL 1991;164:591-2.)

Key words: Fetal acoustic stimulation, fetal panting

Recently, application of ultrasonographic evaluation during or immediately after fetal vibratory acoustic stimulation has been described,[1] leading to further understanding of this fetal-startle response. We describe an unusual case in which marked rapid movement of the fetal chest wall (fetal panting) was seen ultrasonographically after fetal vibratory acoustic stimulation.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Rochester School of Medicine and Dentistry.
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Reprint requests: David M. Sherer, MD, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Strong Memorial Hospital, The University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave., Box 668, Rochester, NY 14642.
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Case report

A 14-year-old black patient was followed during her first gestation at the Strong Memorial Hospital Adolescent Maternity Clinic. Her pregnancy was remarkable for abruptio placentae at 28 weeks' gestation (by an early 6-week ultrasonographic examination), at which time a singleton fetus at the 10th percentile for growth with a small retroplacental hematoma was revealed. She was hospitalized and was discharged 4 days after the vaginal bleeding subsided. Nonstress tests

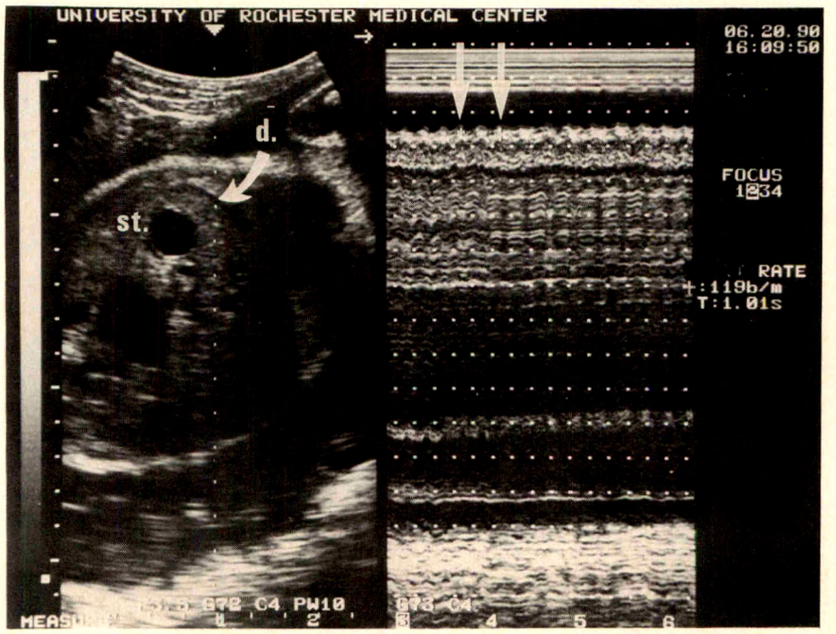


Fig. 1. Split screen ultrasonography. *Left*, M-mode cursor *(dotted line)* traversing anterior fetal chest wall and diaphragm *(d—curved arrow)*, (*st*, stomach). *Right*, M-mode shows fetal panting. *Arrows* mark anterior chest wall movement.

were performed weekly and at 33 weeks' gestation, because of a nonreactive nonstress test, fetal vibratory acoustic stimulation was performed. A reactive response was noted and a biophysical profile was obtained within minutes of the fetal vibratory acoustic stimulation. This revealed an active fetus and 10 of 10 points were given for the biophysical profile. Attention was drawn to persistent, rapid, shallow movement of the anterior fetal chest wall and diaphragm. M-mode ultrasonography with the transducer traversing the fetal diaphragm showed the rate to be 127 movements per minute. The next day, fetal vibratory acoustic stimulation was performed with continuous ultrasonographic scanning. Whereas no fetal chest movement was noted before the fetal vibratory acoustic stimulation, rapid movements of the fetal chest wall and diaphragm at a rate of 119 movements per minute were noted simultaneously after the stimulation. This continued for 40 minutes (Fig. 1). Nonstress tests were performed weekly until term. At 41 weeks' gestation, a normal male infant was delivered vaginally weighing 3330 gm with Apgar scores of 9 and 9 at one and five minutes, respectively. Umbilical artery pH was 7.35, P_{O_2} was 20 mm Hg, and P_{CO_2} was 41 mm Hg. The infant did well. Examination of the placenta did not reveal evidence of placental abruption.

Comment

Recent application of ultrasonography during fetal vibratory acoustic stimulation[1] showed an increase of fetal movements, head turning, mouth opening, tongue protrusion, cheek motion, hand-to-head movement and fetal eye blinking subsequent to such stimulation. These are all thought to represent sequelae of a startle response. In the past, fetal tachypnea has been associated with poor fetal outcome. Devoe et al.[2] reported fetal tachypnea in 18 cases during biophysical tests of 200 patients. The mean breathing rate in their study was 68.2 ± 6.4 breaths per minute. Seven of these 18 fetuses experienced perinatal morbidity. Of these, five had other biophysical abnormalities. Devoe concluded that fetal tachypnea occurs more often than previously believed and does not generally signify fetal compromise unless other biophysical abnormalities are present.

We believe the fetal panting shown in our case (at a rate of twice that noted previously[2]) may be an additional characteristic of the startle response. This case, to the best of our knowledge, represents the first reported occurrence of fetal panting as a response to fetal vibratory acoustic stimulation and, as with the majority of cases described by Devoe, was associated with a normal fetal biophysical profile and good perinatal outcome.

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The increased frequency of cervical dysplasia-neoplasia in women infected with the human immunodeficiency virus is related to the degree of immunosuppression

Axel Schäfer, MD,[a] Wolfgang Friedmann, MD,[a] Martin Mielke, MD,[b] Bernhard Schwartländer, MD,[c] and Meinrad A. Koch, MD[c]

Berlin, Federal Republic of Germany

Cytologic and histologic investigations of the uterine cervix and studies of the lymphocyte functions were performed in human immunodeficiency virus–infected and human immunodeficiency virus antibody–negative women to study possible linkages between human papillomavirus–induced dysplasia and degree of human immunodeficiency virus–induced immunosuppression. Cytologic smears of the uterine cervix of 111 human immunodeficiency virus–infected women were compared with findings in 76 female intravenous drug users negative for human immunodeficiency virus antibodies and in a group of 526 women of the outpatient population of the hospital. Cervical dysplasia-neoplasia (including five cases of invasive carcinoma) was seen in 41% of the human immunodeficiency virus–infected patients. In human immunodeficiency virus–negative intravenous drug users dysplasia-neoplasia was seen in 9%, and in the sample from outpatients in 4%, including two cases of invasive carcinoma ($p < 0.01$). Cytologic features that were attributable to infection with human papillomavirus were observed in human immunodeficiency virus–infected women four times more often than in the sample from the outpatient population ($p < 0.01$). Frequency and severity of dysplasia appear to increase with diminishing numbers of $CD4^+$ helper/inducer T lymphocytes and correlated significantly ($p < 0.01$) with a loss of blastogenic response to phytohemagglutinin, pokeweed mitogen, and tetanus toxoid. These results suggest an increased risk for the development of dysplasia of the uterine cervix in women with human immunodeficiency virus infection, which is related to the degree of immunosuppression. (AM J OBSTET GYNECOL 1991;164:593-9.)

Key words: Cervical dysplasia, HIV infection, immunosuppression, human papillomavirus

The findings that some members of the group of papillomaviruses can transform cells in vitro[1] and the detection of human papillomavirus deoxyribonucleic acid in cells from cervical cancer[2, 3] suggest that human papillomavirus (HPV) may be etiologically involved in the development of cervical dysplasia-neoplasia. An association between HPV and cancer of the cervix has been suggested by epidemiologic studies.[4] A high prevalence of cervical dysplasia among women with human immunodeficiency virus (HIV) infection has been observed[5, 6] and linked to the frequent occurrence of HPV infection in HIV-positive women.[7] Although other well-established cofactors such as sexual activity, frequent genital ulcers, smoking, or use of oral contraceptives[8] must be taken into account, a direct interaction between HIV and HPV on a cellular level or in immunosuppression have been assumed to be additional cofactors.[9]

The frequent occurrence of cervical cancer and cervical intraepithelial neoplasia in immunocompromised women points to a role of immune surveillance in the development of cancer.[10] The infiltration by inflammatory cells, mononuclear cells, and T lymphocytes of HPV-induced lesions in regression is suggestive of a delayed hypersensitivity reaction.[11] An investigation of T-lymphocyte subsets in women with manifest genital HPV infection[12] showed a lower percentage of $CD4^+$ cells (helper/inducer) and a higher percentage of $CD8^+$ cells (cytotoxic/suppressor) compared with women with normal cervical cytologic evaluations. A decrease of the natural killer cell activity could be demonstrated in patients with condyloma acuminatum.[13]

During the course of HIV infection, peripheral T cells and cell-mediated immune response are increasingly diminished.[14] HIV-induced immunodeficiency has been shown to increase not only the risk for opportunistic infections but also the risk for the devel-

From the Department of Obstetrics and Gynecology, Rudolf Virchow Hospital, Free University Berlin,[a] the Department of Immunology, Institute for Microbiology, Free University Berlin,[b] and the Department of Clinical Research, AIDS-Center, Federal Health Office.[c]
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Reprint requests: Axel Schäfer, MD, Special Program for IDU and HIV Infected Women, Department of Obstetrics and Gynecology, Rudolf Virchow Hospital, Free University Berlin, Pulsstr. 4, 1000 Berlin 19, Federal Republic of Germany.
6/1/25053

Table I. Patient characteristics

	Outpatient	*HIV-negative IDUs*	*HIV-positive*
No.	526	76	111
Age (mean ± SD)	27.2 ± 5.5	26.8 ± 4.1	28.9 ± 4.9
Cigarette smoking	223 (42%)	71 (93%)	92 (83%)
Hormonal contraceptives	187 (36%)	0	11 (10%)
Dysplasia-neoplasia*	21 (4%)	7 (9%)	46 (41%)
Invasive carcinoma	2 (0.4%)	0	5 (4%)
Signs of HPV†	61 (12%)	15 (20%)	53 (48%)

IDUs, Intravenous drug users.
*Including invasive carcinoma.
†Koilocytes or dyskaryocytes.

opment of various neoplasms including squamous rectal or oral carcinoma.[15] This poses the question of whether HIV-induced immunosuppression is an important factor in determination of the overt risk of cervical dysplasia and neoplasia in HIV-infected women. To explore these relations peripheral T cells, lymphocyte blastogenic response, and cytologic and histologic characteristics have been investigated in a cohort of HIV-infected women.

Patients and methods

One hundred eleven women, who tested positive for the HIV antibody and who were admitted to this hospital because of various gynecologic complaints or because of an HIV infection that was under regular clinical surveillance, were investigated by cervical cytologic methods. At the same time, parameters of immune functions were determined. Of the 111 HIV-infected women, 84 (76%) had a history of drug use and of sharing injection equipment. The remaining 27 women were presumably infected by sexual contact. The mean age of the patients was 28.9 years (Table I).

Intravenous drug users reported high promiscuity. Many of them had obtained drugs, or money to obtain drugs, by prostitution. Therefore it cannot be excluded that some of the intravenous drug users may have acquired HIV by sexual contact. There were no detectable differences in the frequency of cervical dysplasia-neoplasia or in the clinical or immunologic course of the HIV infection between these patients and patients who reported sexual contact as the only risk for HIV infection.

Results that were obtained in the group of HIV-infected women were compared with findings in a group of 76 women who were addicted to drugs and whose test results were seronegative for HIV. The mean age of this group was 26.8 years. The extent of reported promiscuity and smoking (Table I) was comparable in both groups. Amenorrhea that was caused by opiate use and indolence about possible pregnancies explains the fact that hormonal contraceptives are rarely used by these women.

Results of cervical smears from 526 women who were seen in our outpatient clinic served as controls (mean age, 27.2 years). The vast majority of the women who visited our outpatient clinic did not have regular preventive gynecologic checkups. Minor gynecologic complaints such as itching, vaginal discharge, or lower abdominal pain were the leading causes for consulting the clinic. Because of the location of the hospital, these women represent a population of lower socioeconomic status.

Papanicolaou smears were used for cytologic studies of the uterine cervix. The results were classified as follows: 1 = normal (without signs of dysplasia), 2 = dysplasia (comprising all degrees of dysplasia), and 3 = invasive carcinoma. Cytologic criteria for HPV-induced alterations were the identification of koilocytes and dyskaryocytes in these smears.[16] The investigation was completed by colposcopy. In all patients with colposcopic findings that were suspicious for cervical intraepithelial neoplasia or a cytologic diagnosis of persistent slight or moderate and severe dysplasia, a histologic confirmation by biopsy was obtained.

HIV-induced disease was classified into four groups as proposed by the Centers for Disease Control.[17] This classification relies on clinical findings of HIV-related symptoms. In short, group I included women with acute HIV infection, and group II comprises all HIV-infected women without clinical symptoms. Patients with lymphadenopathy are classified into group III and patients with conditions that were related to acquired immunodeficiency syndrome and acquired immunodeficiency syndrome were assigned to group IV. Groups II and III are subdivided depending on absence or presence of abnormal immunologic findings.

Immunologic studies. Heparinized venous blood was collected for leukocyte counts and functional lymphocyte tests. Lymphocyte subpopulation was determined by flow cytometry with fluorescence-labeled monoclonal antibodies (Coulter) against $CD2^+$ (T lymphocytes), $CD4^+$, and $CD8^+$.

A high individual variability was observed in these tests. To compensate for this variability, mean values were calculated from at least three tests that were performed during an observation period of 6 to 12 months. On the basis of these data, patients were classified into four groups (T1 to T4), which represent increasing stages of $CD4^+$ cell depletion. They were distinguished as follows: T1—no detectable changes in the number of $CD2^+$ lymphocytes, $CD4^+/CD8^+$ ratio >1; T2—$CD4^+/CD8^+$ ratio <1, $CD4^+$ cell counts >400/μl; T3—$CD4^+$ cell counts between 400 and 250/μl; T4—$CD4^+$ cell counts <250/μl.

Table II. Correlation of cytologic signs of HPV* to dysplasia/neoplasia

	No signs of dysplasia	*Dysplasia-neoplasia*	*Total*
Outpatients	54/505 (11%)	7/21 (33%)	61/526 (12%)
HIV-negative IDUs	11/69 (16%)	4/7 (57%)	15/76 (20%)
HIV-positive	17/65 (26%)	36/46 (78%)	53/111 (48%)

IDUs, Intravenous drug users.
*Koilocytes or dyskaryocytes.

Lymphocyte functions were determined with a whole-blood lymphoproliferation assay as described previously.[18] In short, heparinized venous blood was diluted 1:8 in the *synthetic culture medium* RPMI 1640 (Biochrom), which was supplemented with penicillin, streptomycin, and glutamine. Subsequently 100 μl of this suspension was added to 100 μl dilutions of phytohemagglutinin (20, 10, and 5 μg/ml), pokeweed mitogen (20, 10, and 5 μg/ml), and tetanus toxoid (10 and 5 μg/ml) in U-bottom microtiter plates. Cultures were incubated for 5 days. Proliferative responses were determined by measuring the amount of incorporated tritiated thymidine after labeling for 5 hours. For each mitogen or antigen concentration, determinations were made in triplicate. Results from 240 healthy women served to determine baseline values. As an indication of an impaired proliferative response, only significantly decreased values of tritium incorporation below the 5th percentile of the healthy controls were considered. Titers of antitetanus antibodies were determined with an enzyme immunosorbent assay (detection limit: 0.15 units of antitoxin per milliliter).

On the basis of the data that were obtained, patients were classified into five groups (M1 to M5), which represent increasing stages of deficiencies in the lymphoproliferative response. This classification was developed on the basis of long-term observations of more than 200 HIV-infected individuals (unpublished observations). The different stages are characterized by: M1—normal blastogenic response to phytohemagglutinin, pokeweed mitogen, and tetanus toxoid, ≥0.3 U/ml of antitetanus toxoid antibodies in the serum; M2—decrease of lymphocyte responsiveness to tetanus toxoid only; M3—as M2 and decrease of lymphocyte responsiveness to pokeweed mitogen; M4—as M3 and decreased titers of circulating antibodies against tetanus toxoid <0.3 U/ml; and M5—as M4 and decrease of phytohemagglutinin-induced lymphocyte transformation.

Results

Cytologic evaluation of the uterine cervix in the investigated collectives. Between August 1985 and February 1989, 111 HIV-infected women were investigated. The shortest period of observation was 6 months. The clinical investigation included a stage determination of HIV disease, cytologic examination of the uterine cervix, histologic examination when indicated, and investigations of the immune functions.

Of these patients 46 (41%) had cytologic smears that were suspicious for cervical dysplasia/neoplasia including five cases of invasive carcinoma (Table I). A consistently normal cytologic evaluation during the time of observation was found in 65 (59%) of the 111 HIV-infected women. In 12 cases progression of dysplasia and in 11 cases spontaneous regression of dysplasia could be observed.

Of the 76 HIV-negative women who were addicted to drugs 69 (91%) exhibited a normal cytologic evaluation, and only seven (9%) had dysplasia. Invasive carcinoma was not seen in this group. The screening results for abnormal cytologic smears of the uterine cervix in women who visited our outpatient service are included in Table I. Normal cytologic smears were obtained from 505 (96%) of 526 women, and 21 (4%) showed varying degrees of dysplasia/neoplasia including two cases with histologically confirmed invasive carcinoma.

Dysplasia was seen in the group of HIV-infected women about four times more often than in the group of intravenous drug users without HIV infection ($p < 0.01$) and about 10 times more often than in the outpatient population ($p < 0.01$).

In the group of HIV-negative female intravenous drug users dysplasia was twice as frequent as in the outpatient population ($p = 0.02$). Signs for HPV infection (koilocytes and dyskaryocytes) were seen significantly more often in HIV-infected women (Table II).

Colposcopy and histologic diagnosis in HIV-infected women. By colposcopy 83% (38/46) of the HIV-infected patients with dysplasia were conspicious for cervical intraepithelial neoplasia. Thirty-two percent (14/46) had additional alterations that were attributed to HPV, such as condylomas. Biopsy specimens of uterine cervix were obtained from 38 HIV-infected women. In addition to the histologic confirmation of five invasive carcinomas, cervical intraepithelial neoplasia III was seen in 13 cases, cervical intraepithelial

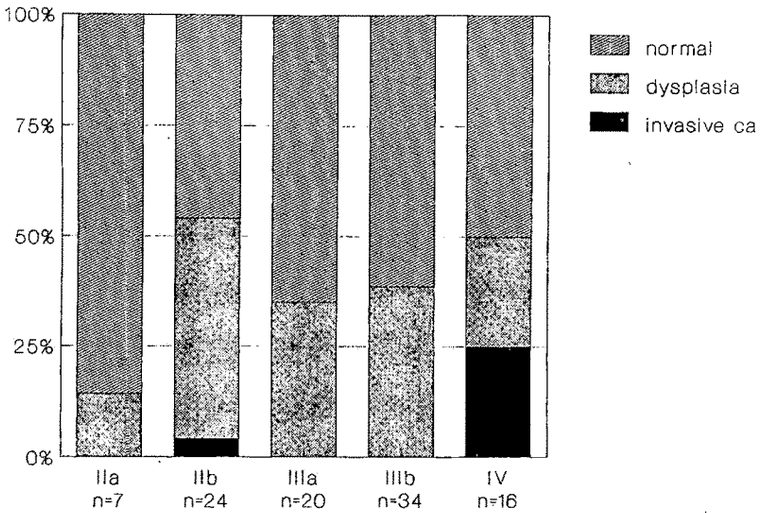


Fig. 1. Centers for Disease Control classification of HIV disease and cervical dysplasia.

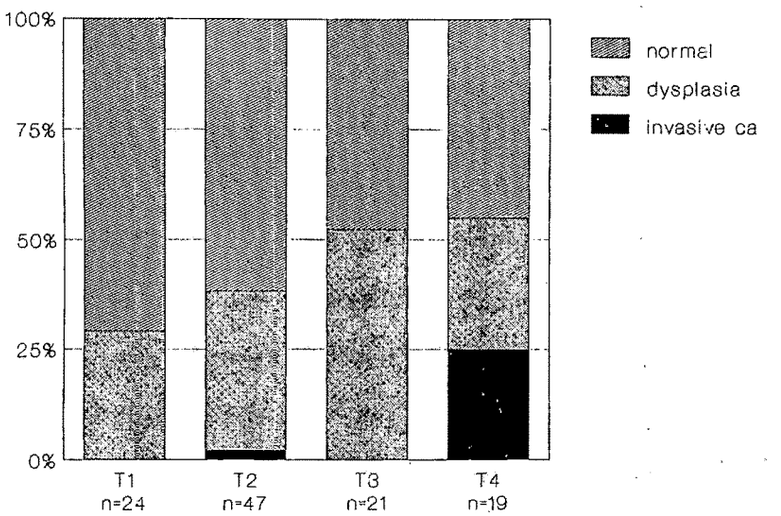


Fig. 2. Cervical dysplasia and neoplasia in correlation to $CD4^+$ lymphocyte depletion in HIV-infected women.

neoplasia II in 11 cases, cervical intraepithelial neoplasia I in 7 cases, and cervicitis in 2 cases. Depending on the histologic severity of cervical intraepithelial neoplasia, 21 women underwent conization. One hysterectomy and three Wertheim-Meigs operations had to be performed. The International Federation of Gynecology and Obstetrics staging of the carcinomas was in two cases stage IA, in one case stage IIA, in one case stage IIB, and in one case stage III with an additional vulvar carcinoma.

Relation of cervical cytologic evaluation to clinical and immunologic status of HIV infection. Nearly 50% of the women with HIV-seropositive test results had generalized lymphadenopathy together with abnormal immunologic findings and were therefore classified as Centers for Disease Control stages IIIB and IV. Although four out of the five carcinomas that were detected were seen in patients with stage IV HIV disease ($p < 0.01$), there was no significant correlation of the frequency of dysplasia to the different stages of HIV disease (Fig. 1).

To define immunosuppression more precisely, T-cell

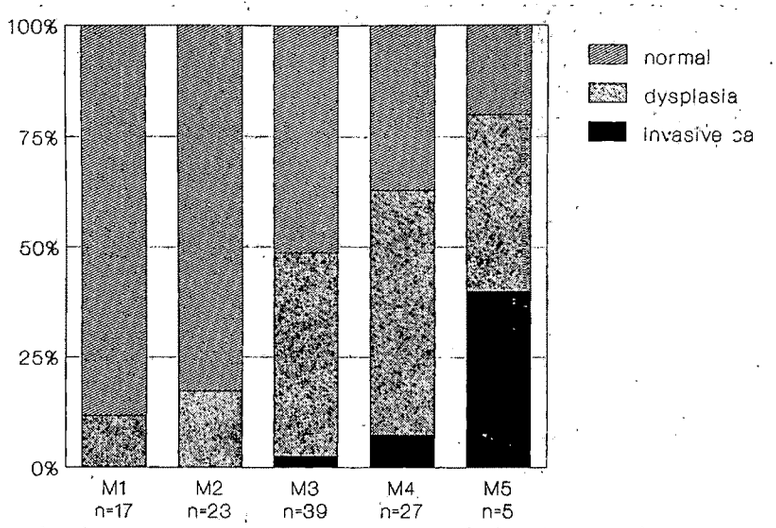


Fig. 3. Cytologic findings in HIV-infected women with defined defects in the lymphoproliferative response.

Table III. Cytologic findings in HIV-infected women with defined alterations of lymphocyte subsets

	No signs of dysplasia	*Dysplasia*	*No. with invasive carcinoma*	*Total*
T1	17 (70%)	7 (30%)		24
T2	29 (62%)	18 (38%)	1	47
T3	10 (48%)	11 (52%)		21
T4	9 (47%)	10 (53%)	4	19
TOTAL	65 (59%)	46 (41%)	5	111

Table IV. Cytologic findings in HIV-infected women with defined defects in the lymphoproliferative response

	No signs of dysplasia	*Dysplasia*	*No. with invasive carcinoma*	*Total*
M1	15 (88%)	2 (12%)		17
M2	19 (83%)	4 (17%)		23
M3	20 (51%)	19 (49%)	1	39
M4	10 (37%)	17 (63%)	2	27
M5	1 (20%)	4 (80%)	2	5
TOTAL	65 (59%)	46 (41%)	5	111

subset counts were performed, and parameters of lymphocyte function were determined. The frequency of dysplasia-neoplasia at defined alterations of T-cell subsets is shown in Fig. 2 and Table III. Again four of the five carcinomas that were detected were seen in patients with the most pronounced $CD4^+$ cell depletion (>250/μl) ($p < 0.01$). The apparent correlation of dysplasia and lymphocyte depletion is statistically not significant ($p = 0.2$) because of the small number of patients. However, a highly significant correlation of cervical dysplasia or invasive carcinoma with functional immunosuppression as determined by assays of lymphocyte proliferation (M1 to M5) could be demonstrated ($p < 0.01$; Fig. 3 and Table IV).

Comment

In this study the frequency of cervical dysplasia-neoplasia and signs of HPV infection were compared in three groups of women of similar age. Baseline data that were obtained in 526 women from the outpatient

population were related to data that were obtained from HIV-infected women and HIV-negative female intravenous drug users. The two latter groups were equally promiscuous and therefore had a comparable risk of HPV infection.

HIV-infected women had a tenfold higher prevalence of dysplasia-neoplasia than the outpatient population and a still fourfold higher prevalence of dysplasia-neoplasia than HIV-negative female intravenous drug users.

Several findings point to a role of HPV in the cause or development of cervical dysplasia-neoplasia.[19-21] The reported high prevalence of latent HPV infection in populations of healthy women suggests that HPV infection by itself may not be sufficient to cause the development of cervical dysplasia or cancer.[22] The increased manifestation of active HPV infection as proved by the presence of koilocytes or dyskaryocytes in 81% of HIV-infected patients with dysplasia-neoplasia suggests an HIV-induced impairment of cellular immune response. In addition to established cofactors such as sexual activity, frequent genital infections, and smoking habits, which are frequently found in this group, we propose HIV-induced immunosuppression as another important cofactor in the risk of dysplasia/neoplasia. The observation that HIV-infected but immunocompetent women showed a prevalence of dysplasia/neoplasia that was comparable to the prevalence seen in HIV-negative women with a comparable risk for HPV infection argues against a mere synergistic interaction between HIV and HPV, as suggested for herpes simplex viruses and HIV.[23]

In our study the impairment of lymphocyte functions was significantly correlated with an increasing risk for cervical dysplasia/neoplasia. A comparable explanation has been proposed by other authors who found an increased prevalence of cervical cancer in women who were immunocompromised by Hodgkin's disease[24] or who had iatrogenic immunosuppression after renal transplantation.[25]

These interpretations are supported by studies that demonstrate that in animal models cell-mediated immune responses directed against viral or virally induced antigens represent the major effector mechanism in the control of papillomavirus infections.[26] It is therefore tempting to speculate that the HIV-induced immunodeficiency may lead to an active chronic HPV infection and thereby to persistence or progression of dysplasia. The correlation of cervical dysplasia and invasive carcinoma to the degree of immunologic impairment in HIV-infected women raises the question of whether HPV-induced dysplasia/neoplasia should be considered as an opportunistic disease.

As a consequence, clinical surveillance of HIV-infected women should include cytologic investigation of the uterine cervix at regular intervals; the frequency depends on the stage of HIV disease. The high proportion of suspicious colposcopic findings and the observed severity of cervical intraepithelial neoplasia in HIV-infected women demand careful colposcopic examinations and immediate directed biopsy when indicated. This is especially important in intravenous drug users who show a low compliance with the need for regular medical checkups.

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Characterization of enzymes of catecholamine synthesis and metabolism in human fetal membranes at birth

Viviane Casimiri, PhD,[a] Germaine Acker, PhD,[b] Simone Parvez, PhD,[a] Hasan Parvez, PhD,[a] Lony Castro, MD,[c] Calvin Hobel, MD,[c] and Emile Papiernik, MD[b]

Orsay and Clamart, France, and Los Angeles, California

We looked for the presence of the enzymes monoamine oxidase, catechol-O-methyltransferase, and phenylethanolamine-N-methyltransferase in human fetal membranes at term. The activity of all three enzymes was detected via highly sensitive and selective radiometric enzyme assays. The most novel finding was the extremely high level of monoamine oxidase activity in the chorion compared with that in the amnion. The other enzyme of catecholamine metabolism, catechol-O-methyltransferase, did not show any difference in activity between the two layers. In addition, we observed that the enzyme phenylethanolamine-N-methyltransferase, which is primarily located in the adrenal medulla, was also present in appreciable levels in the two layers of fetal membranes. These results suggest that fetal membranes, like the placenta, possess the enzymatic machinery to metabolize catecholamines and have the capacity to synthesize epinephrine. (Am J Obstet Gynecol 1991;164:599-603.)

Key words: Enzymes for synthesis and metabolism, catecholamines, chorion, amnion

The participation of adrenergic innervation in the maturation of fetal development has been looked on critically by several groups.[1, 2] Numerous studies have analyzed extensive biochemical and neuroendocrine factors that can lead to fetal delivery at term.[3] The focus of these studies has been the importance of the uterus and the placenta. Neurochemical exploration of the placenta suggests that this organ contains most of the inactivating enzymes necessary to deliver physiologic concentrations of different transmitters and hormones. It has also been established that placental tissue possesses significant levels of two enzymes, monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT), which are involved in catecholamine metabolism.[4] Fig. 1 depicts a general overview of catecholamine biosynthesis and metabolism. The significance of MAO and COMT in the inactivation of catecholamines from the maternal circulation can be shown by perfusion of labeled catecholamines to the mother and their detection in the fetus.[5] Placental tissue apparently serves as a barrier to prevent excessive levels

From the Unite de Neuropharmacologie, Universite Paris XI, Centre d'Orsay,[a] the Maternite Antoine Beclere, Universite Paris XI and INSERM U-187, Hopital Antoine Beclere, Clamart,[b] and the Division of Maternal-Fetal Medicine,[c] Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, University of California, Los Angeles, School of Medicine.
Received for publication October 27, 1989; revised June 7, 1990; accepted August 31, 1990.
Reprint requests: Viviane Casimiri, PhD, Unite de Neuropharmacologie, Universite Paris XI, Centre d'Orsay, Bat 440, 91405 Orsay Cedex, France.
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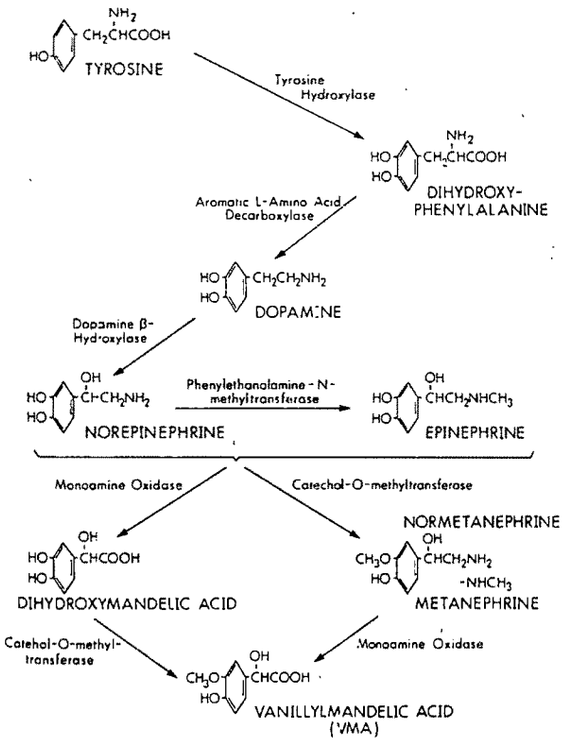


Fig. 1. Catecholamine biosynthesis and metabolism. (Reproduced from the *Cecil Textbook of Medicine*, 18th edition, 1988. Reprinted with the permission of W.B. Saunders Co.)

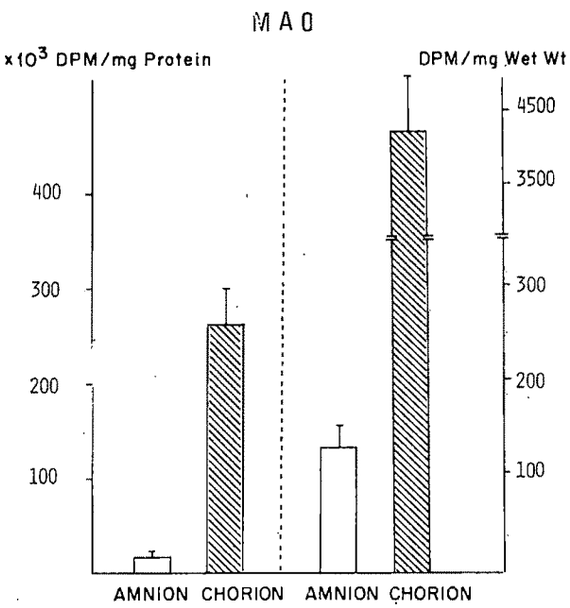


Fig. 2. Activity of enzyme MAO in two layers of fetal membranes. Activity has been expressed per milligram protein, as well as per milligram wet weight. MAO activity was significantly greater in chorion than in amnion ($p < 0.001$, $n = 10$).

of norepinephrine and epinephrine from crossing the maternal circulation to the fetus.

The presence of catecholamines and their metabolites in fetal amniotic fluid has been observed in our laboratory, as well as by several other authors. These studies indicated that levels increase with gestational age and labor.[6,7] Studies by Phillippe et al.[8] support a role for the fetal membranes in the metabolism of catecholamines. In addition, our recent studies show a strong modulation of prostaglandin secretion by fetal membranes in response to catecholamine stimulation.[9] However, according to our knowledge, the neurochemical characterization of the different enzymes of catecholamine metabolism and synthesis in fetal membranes has not been previously reported. The goal of the present investigation was to identify the presence of the catecholamine metabolizing enzymes (MAO and COMT) and the synthesizing enzyme phenylethanolamine-N-methyltransferase (PNMT) in the human chorion and amnion.

Material and methods

This study was approved by the institutional review board of the Maternite Hopital Antoine Beclere of the University of Paris. Human fetal membranes (chorion and amnion) were obtained at the time of cesarean section from 10 women in labor at term. The membranes were rapidly dissected into chorion and amnion and were stored at −80° C until assayed. Both the amnion and the chorion were homogenized in isotonic potassium chloride at a concentration of 400 mg/ml. The homogenate for MAO assays was further diluted 10 times with isotonic potassium chloride. One part of the concentrated homogenate was centrifuged at 35,000 *g* for 30 minutes at 4° C for the preparation of cytosol for the assays of the enzymes PNMT and COMT.[10]

MAO assay. The total activity of MAO was determined by use of tryptamine-^{14}C-bisuccinate as substrate (specific activity, 49.6 mCi/mmol, New England Nuclear, Boston). The assay was adapted to be performed in disposable 1 ml polypropylene microtubes, and its sensitivity was increased. The incubation mixture consisted of 100 μl of phosphate buffer (0.1 mol/L, pH 7.4), 50 μl of diluted tissue homogenate (40 mg/ml), and 50 μl of tryptamine-^{14}C-bisuccinate (1 nmol). The tubes were incubated for 20 minutes at 37° C. The reaction was stopped by transferring the tubes to an ice bath and by the addition of 20 μl of 2N hydrochloric acid and 600 μl of toluene. The assay tubes were shaken for 20 minutes on a mechanical shaker and centrifuged at 3000 *g* for 10 minutes. The top layer (400 μl) was separated with a micropipette and transferred to a miniscintillation vial containing 1.5 ml of toluene scintillation liquid. The extracted disintegrations per min-

ute represented a direct linearity with the nanomoles of ^{14}C-indole acetic acid transformed in 20 minutes.[11]

COMT assay. The enzyme COMT was determined with the use of S-adenosylmethionine-^{3}H-methyl (Center Etudes Atomic, Gif Sur/Yvette, France) as a cofactor for O-methylation of catecholamines. Again, the assay was adapted to be performed in 1 ml polypropylene disposable tubes.[12] The incubation mixture consisted of 100 μl of phosphate buffer (0.1 mol/L, pH 7.9); 10 μl of 1% magnesium choride; 50 μl of tissue supernatant as described above (400 mg/ml), 50 μl (1.11 nmol) of S-adenosylmethionine-^{3}H-methyl (45 mCi/mmol), and 10 μl of epinephrine (11.6 mg/ml). The tubes were incubated at 37° C for 1 hour. The reaction was stopped by the transfer of the tubes to an ice bath and by addition of 50 μl of 1 mol/L borate buffer at pH 10.0. Methylated products were separated by the addition of 600 μl of toluene + isoamyl alcohol (3:2) and shaking for 20 minutes. The tubes were centrifuged at 3000 *g* for 10 minutes, and 400 μl of the organic phase of the solution was extracted by a micropipette and added to 1.5 ml of scintillation solution in a minivial. The disintegration per minute represented a direct linearity with the nanomoles of methylepinephrine that formed in 1 hour.[12]

PNMT assay. The PNMT assay was very similar to that of COMT, except that magnesium chloride was not added. The substrate used was normetanephrine (50 μl of a 2 mg/ml solution). The time of incubation was 1 hour, as it was for COMT. The extraction procedure was the same as that used for COMT, except that 440 μl of organic phase was extracted. The details of the assay have been provided elsewhere.[12, 13] The extracted radioactivity was directly linear to the nanomoles of metanephrine formed in 1 hour.

The results were subjected to statistical analysis by the paired Student *t* test. The values are expressed as the mean ± SEM. A p value <0.05 was considered significant.

Results

Fig. 2 illustrates the total activity of the enzyme MAO in amnion and chorion layers of the fetal membranes. Enzyme activity was extremely low in the amnion layer, regardless of how the results were expressed. MAO activity in the chorion layer was 16-fold (per milligram protein) and 31-fold (per milligram wet weight) higher than that in the amnion layer. These elevations were highly significant ($p < 0.001$).

Fig. 3 shows the presence of activity of the enzyme COMT in the two layers of human fetal membranes. No significant difference was observed between enzyme activity in the chorion and amnion when the results were expressed per milligram protein. However, expressing COMT activity per milligram wet weight of the two layers showed a 2.3-fold higher level in the chorion than in the amnion; this difference was significant ($p < 0.05$).

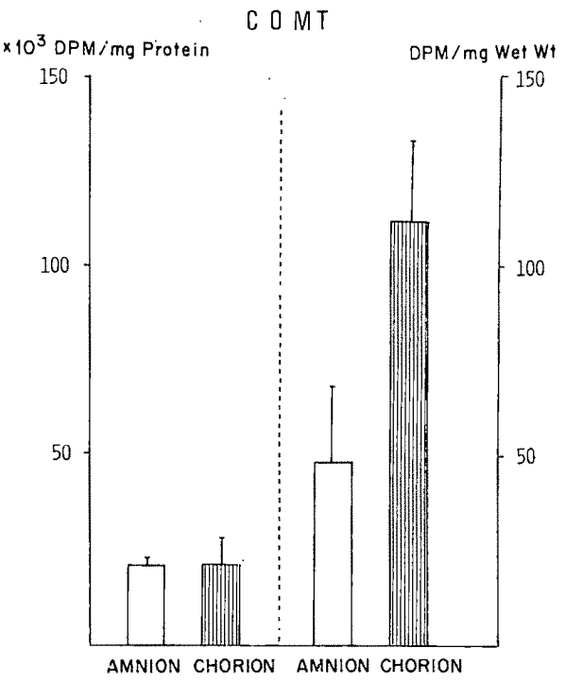


Fig. 3. Enzyme COMT in two layers of fetal membranes. COMT activity per milligram wet weight was greater in chorion than in amnion ($p < 0.05$, $n = 10$).

Fig. 4 shows the level of the enzyme PNMT in the two different layers of human fetal membranes. In the adult this enzyme is primarily confined to the adrenal medulla, but some traces can be found in other tissues. It was interesting to observe that both the amnion and the chorion contain PNMT and therefore possess the enzymatic machinery to form epinephrine endogenously. Contrary to the enzymes of catecholamine metabolism, PNMT activity in the chorion was not found to be statistically different from that in the amnion.

Comment

The results presented in this study show that the two layers of the human fetal membranes possess the enzymatic machinery to metabolize and synthesize catecholamines endogenously. These findings strengthen the hypothesis suggested by Zuspan et al.[14] in 1981, which suggests that adrenergic transmitters may play a role in parturition. The existence of the enzymes MAO and COMT in the chorion and amnion may be of great functional importance because norepinephrine and epinephrine can pass through the placental barrier and appreciable amounts of these transmitters can be localized in the amniotic fluid of human fetuses.[7, 15]

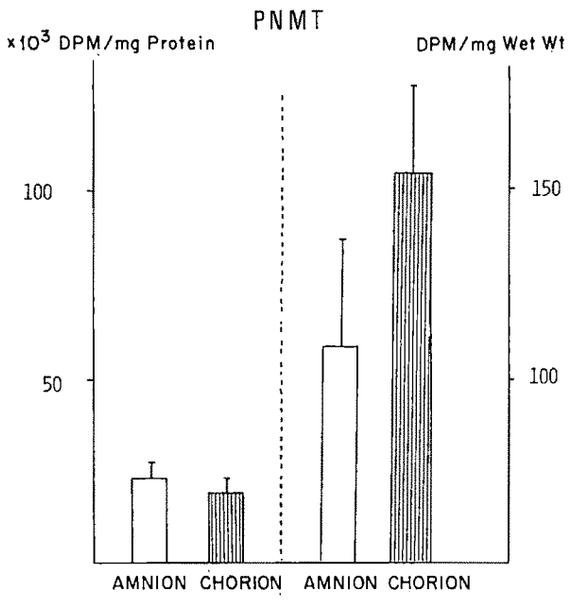


Fig. 4. Activity of enzyme PNMT in amnion and chorion layers of fetal membranes (p = NS, n = 10).

Higher concentrations of catecholamines entering the fetal circulation could be hazardous for normal fetal growth and development. On the basis of our data, it appears that the fetal membranes are equipped to exert oxidative deamination of both intracellular catecholamines and of O-methylation of circulating biogenic amines that could otherwise enter the fetal circulation. Such a process at the membrane level may provide extra protection to the preterm fetus.

The most novel finding of this investigation is the tremendous difference in the activity of the enzyme MAO in the chorion and amnion. A high content of MAO in the chorion suggests that intracellular oxidative deamination may be the most important pathway of transmitter inactivation in the chorion layer of fetal membranes.

Biochemical studies performed in vitro also suggested that the human chorioamnion has the capacity to rapidly metabolize labeled dopamine to dihydroxyphenylacetic acid, 3-methoxy,4-hydroxyphenylacetic acid, and 3-methoxy,4-hydroxyphenylethanol.[8] Phillippe and Niloff[8] speculated that the presence of catecholamine metabolizing enzymes in the chorioamnion could be responsible for the rapid conversion of labeled dopamine to different metabolites.

This study supports these conclusions. Another study that shows an increase in catecholamines and metabolites in the amniotic fluid compartment from middle to late gestation agrees with our data on the localization of MAO and COMT in the chorion and amnion layers of human fetal membranes.[7] It is necessary to characterize the different forms of the enzyme MAO in the chorion and amnion to identify specific participation of different metabolic pathways in each layer of the fetal membranes in early and late pregnancy. Such a study could clarify the results obtained in vitro with irreversible inhibitors of MAO.[8] The use of clorgyline (an MAO-A inhibitor) and deprenil (an MAO-B inhibitor) can distinguish the physiologic participation of dopamine from that of norepinephrine or epinephrine. Normally, the human fetal placenta contains only MAO-A and the placenta has very close structural similarities to the chorioamnion since both these tissues lack innervation. Therefore elucidation of the specific pathway involved in catecholamine metabolism could be useful in understanding the effects of catecholamines on fetal well-being and development.

The existence of PNMT in the amnion and chorion at trace levels suggests that fetal membranes have the capacity to undergo N-methylation of norepinephrine to epinephrine. This process may be useful in controlling the vasoactive response that could develop during excessive placental transport of catecholamines to the fetal circulation. The formation of epinephrine by fetal membranes may also modulate prostaglandin secretion, thus providing a stimulus for the onset of labor.[9]

In summary, the results of this study indicate that human fetal membranes have the capacity to metabolize and synthesize catecholamines. The most interesting finding is the large difference in MAO activity between the chorion and amnion. Isolation of the specific forms of MAO present in the amnion and chorion at different stages of gestation is necessary for interpretation of the role of this enzyme during fetal growth and development and during labor.

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Pregnancy-related mortality in New York City, 1980 to 1984: Causes of death and associated risk factors

Carla J. Syverson, CNM, MN, MPH,[a, d] Wendy Chavkin, MD, MPH,[b] Hani K. Atrash, MD, MPH,[c] Roger W. Rochat, MD,[a] Elizabeth S. Sharp, CNM, DrPH,[d, e] and Gail E. King, MD, MPH[a]

Atlanta, Georgia, and New York, New York

To identify causes and risk factors for pregnancy-related mortality in New York City, we analyzed 224 pregnancy-related deaths that occurred from 1980 to 1984. The leading causes of death were ectopic pregnancy complications, embolism, intrapartum cardiac arrest, and hypertension. Mortality ratios were determined by comparing the characteristics of the women whose death was pregnancy-related with those of women who had survived delivery of a live infant in New York City during the same period. Black and Hispanic women had mortality ratios that were respectively 4.2 and 2.0 times higher than those for white, non-Hispanic women. In comparison with women aged 20 to 24, those older than 30 were more than twice as likely to die from pregnancy-related causes, and those older than 40 were five times as likely to do so. Other factors that were associated with an increased risk of pregnancy-related mortality included 9 to 11 years of education, lack of private medical insurance, more than five previous pregnancies, and fewer than five prenatal visits. This study suggests that changes in current maternal-health and family-planning services will be required to achieve further reductions in preventable pregnancy-related mortality. (Am J Obstet Gynecol 1991;164:603-8.)

Key words: Pregnancy, embolism, ectopic pregnancy, anesthesia

Since 1915, the pregnancy-related mortality ratio (pregnancy-related deaths per 100,000 live births) has declined in the United States, from 700 in 1915[1] to 7.8 in 1984.[2] Although this overall decline has been dramatic (approximately 90% over 70 years), there has been an insignificant decrease since 1980.[2-6]

Pregnancy-related mortality ratios in the northeastern United States and in countries such as Ireland, the Netherlands, Scandinavia, and Switzerland have been reduced to between 4 and 5 per 100,000 live births.[7] Several researchers describe significant underreporting of cases and have found the actual rate of pregnancy-related mortality to be from 39% to 112% above that reported,[8-13] which would place the true United States pregnancy-related mortality ratio between 10.8 and

From the Master of Public Health Program, Emory University,[a] the New York City Department of Health,[b] the Division of Reproductive Health, Centers for Disease Control,[c] the Department of Gynecology and Obstetrics, the School of Medicine, Emory University,[d] and the Nell Hodgson Woodruff School of Nursing, Emory University.[e]
Received for publication January 31, 1990; revised August 14, 1990; accepted August 31, 1990.
Reprint requests: Carla Syverson, CNM, MN, MPH, c/o Hani Atrash, MD, MPH, Centers for Disease Control, Division of Reproductive Health, 1600 Clifton Road, NE, Bldg. 1, Room 4409, Atlanta, GA 30333.
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Table I. Pregnancy-related mortality ratios, New York City and United States, 1980 to 1984*

Year	New York City		United States (ratio)
	No. of deaths	Ratio	
1980	55	51.4	9.2
1981	44	40.5	8.5
1982	47	42.2	7.9
1983	50	43.6	8.0
1984	28	24.7	7.8
1980–1984	224	40.2	8.2

*Pregnancy-related deaths per 100,000 live births.

16.5. One of the 1990 objectives of the United States Public Health Service is to achieve a pregnancy-related mortality ratio of 5 deaths per 100,000 live births.[14]

The major causes of pregnancy-related deaths in the United States have been attributed to the classic triad of hemorrhage, pregnancy-induced hypertension, and infection.[15] More recent studies indicate a change in this pattern, because embolism, ectopic pregnancy complications, and cardiomyopathy account for increasingly larger proportions of pregnancy-related deaths.[11, 12, 16, 17]

Risk factors for pregnancy-related death have received little attention in the literature. Previous studies have consistently shown minority women to have a pregnancy-related mortality ratio that is three to four times higher than that of white women.[11, 16-18] Kaunitz et al.[16] found women older than 30 to be at greater risk for pregnancy-related death than are younger women.

In this article we present the results of an investigation of all pregnancy-related deaths reported in New York City from 1980 through 1984 in different subgroups of women, and we describe mortality ratios, causes of death, and risk factors for pregnancy-related mortality.

Material and methods

The Bureau of Maternity Services and Family Planning of the New York City Department of Health has long been studying pregnancy-related mortality. Pregnancy-related deaths in New York City are reported to the New York City Department of Health, where a file is maintained on each death. Complete files contain copies of the death certificate, the certificate of live birth or a certificate of termination of pregnancy, the autopsy report, the medical records, and a case summary by the Bureau's obstetric consultant. We collected data for this study by reviewing each file.

For this study, a pregnancy-related death was defined as the death of any woman, from any cause, while she was pregnant or was within one calendar year of termination of pregnancy, regardless of the length and site of pregnancy. Deaths were classified as resulting from direct, indirect, or other causes. A direct pregnancy-related death was defined as a death that resulted from the management or condition of pregnancy, labor, or delivery. An indirect pregnancy-related death was defined as a one in which pregnancy exacerbated a preexisting health condition. A death that resulted from other causes was defined as a death that occurred during pregnancy or within 1 year after the termination of pregnancy but was not considered direct or indirect (for example, death from homicide or drug overdose). We use the term pregnancy-related rather than maternal because the latter term means *pertaining to the mother,*[19] and our descriptions of deaths include deceased women, such as those who had an ectopic pregnancy or abortion, whose pregnancy did not result in birth.

Pregnancy-related mortality ratios for New York City and for the United States were calculated from vital statistics provided by the New York City Department of Health[20-24] and by the National Center for Health Statistics (Table I).[2-6]

The cause of death was determined from the autopsy report or from the medical record if no autopsy report was available. In the absence of other records the cause of death was taken from the death certificate.

All deaths were attributed to one of the causes listed in Table II. Deaths attributed to ectopic pregnancy complications included all deaths involving an ectopic pregnancy, such as mortality from anesthesia complications that occurred during surgery for ectopic rupture. Deaths from embolism included both thromboemboli and emboli caused by amniotic fluid. Deaths attributed to intrapartum cardiac arrest included complications of anesthesia (not those associated with ectopic pregnancy or elective abortion) and all deaths that resulted exclusively from cardiac arrest during cesarean section without mention of other conditions or complications. Hypertension as the cause of death included both preexisting and pregnancy-induced hypertension; only two women in the study were known to have preexisting hypertension.

We examined the following risk factors to determine their association with pregnancy-related death: age at the time of death, race, marital status, education, number of previous live births, number of prenatal visits, and source of payment for health services. The analysis was limited to these factors, which included all the information that could be obtained from the comparison group of women who survived after delivery of a live infant in New York City from 1980 to 1984. We used Woolf's method to calculate relative risks with 95% confidence intervals.[25]

Missing or unknown data for the pregnancy-related

Table II. Causes of pregnancy-related death, New York City, 1980 to 1984

Cause of death	*No.*	*%*	*Ratio**
Direct cause			
Ectopic complications	30	13.4	5.4
Embolism	29	12.9	5.2
Hypertension (including pregnancy-induced hypertension)	23	10.3	5.1
Cardiac arrest (intrapartum)	19	8.5	3.4
Cerebrovascular accident	17	7.6	3.1
Induced abortion complications	14	6.2	2.5
Hemorrhage	7	3.1	1.3
Disseminated intravascular coagulopathy	6	2.6	1.1
Obstetric infection	5	2.2	0.9
Postpartum cardiomyopathy	4	1.8	0.7
Anesthesia complications	4	1.8	0.7
Spontaneous abortion complications	3	1.3	0.5
Liver disease of pregnancy	2	0.9	0.4
Other obstetric surgical complications	2	0.9	0.4
SUBTOTAL	165	73.5	
Indirect cause			
Nonobstetric infection	15	6.7	2.7
Preexisting cardiovascular disease	9	4.0	1.6
Sickle cell crisis	5	2.2	0.9
Systemic lupus erythematosus	3	1.3	0.5
Cancer	2	0.9	0.4
Acquired immunodeficiency syndrome	2	0.9	0.4
Degenerative central nervous system disease	1	0.4	0.2
SUBTOTAL	37	16.4	
Other cause			
Homicide	7	3.1	1.3
Suicide	5	2.2	0.9
Drug overdose	3	1.3	0.5
Asphyxia by accidental fire	2	0.9	0.4
Anesthesia during tubal ligation	1	0.4	0.2
SUBTOTAL	18	7.9	
Unknown cause	4	1.7	0.7
TOTAL	224	99.5	

*Pregnancy-related deaths per 100,000 live births.

deaths and for the live births of the comparison group were assigned values according to the frequency distribution of the known data. For example, the distribution of known data for means of payment was 18.8% self-pay, 40.9% Medicaid, and 40.3% third-party. The 75 pregnancy-related deaths for which means of payment data were missing were randomly distributed so that 18.8% (n = 14) were assigned to self-pay, 40.3% (n = 31) were assigned to Medicaid, and the remaining 40.3% (n = 30) were classified as third-party.

Live-birth data were reported with at least 90% completeness for each variable. Age, race, and marital status were reported for at least 90% of the pregnancy-related deaths. Data on education, gravidity, parity, prenatal care, and source of payment were reported in varying frequencies of >50%.

Results

Despite a decrease, which probably resulted from a reporting lag, to 24.7 in 1984, per 100,000 live births, the overall pregnancy-related mortality ratio for New York City for the years 1980 to 1984 was 40.2 deaths per 100,000 live births (Table I). Whereas the greatest number of deaths and the highest ratio were seen in 1980, little variation in these factors was noted from 1981 to 1983. The average pregnancy-related mortality ratio for New York City during 1980 to 1984 was approximately five times greater than that reported for the entire United States.

Most causes of pregnancy-related death (73.5%) were classified as the direct result of complications during pregnancy, the most frequent of which were ectopic pregnancy (13.4%), pulmonary emboli (12.9%), hyper-

Table III. Pregnancy-related mortality ratios by risk factor, New York City, 1980 to 1984 (n = 224)

Risk factor	*No. of deaths**	*Ratio†*	*Relative risk*	*95% Confidence interval*	*Completeness of reporting (%)*
Age (yr)					99.6
14-19	25	36.0	1.30	0.79-2.14	
20-24	41	27.8	1.00		
25-29	54	32.2	1.16	0.77-1.74	
30-34	67	57.2	2.09	1.42-3.08	
35-39	27	63.2	2.20	1.40-3.71	
≥40	10	133.1	4.79	2.40-9.57	
Race					98.2
White	31	15.6	1.00		
Black	122	70.5	4.23	2.85-6.28	
Hispanic	63	39.9	2.00	1.34-3.24	
Other	8	33.2	2.06	0.95-4.48	
Married					96.4
Yes	112	31.8	1.00		
No	112	55.7	1.75	1.35-2.27	
Years of education					50.4
0-8	16	37.4	1.37	0.73-2.55	
9-11	63	53.5	1.93	1.22-3.05	
12-14	119	40.0	1.45	0.95-2.22	
≥15	26	27.3	1.00		
Source of payment					66.5
Self	42	69.0	2.33	1.62-3.36	
Medicaid	92	49.0	1.66	1.24-2.22	
Third party	90	29.6	1.00		
Previous live births					77.2
0	82	32.0	1.00		
1	63	39.7	1.23	0.89-1.71	
2	35	44.8	1.39	0.94-2.06	
3-4	31	66.5	2.06	1.36-3.11	
≥5	13	94.4	2.94	1.64-5.28	
Previous pregnancies					77.7
0-4	192	36.5	1.00		
≥5	32	115.9	3.15	2.17-4.58	
No. of prenatal visits					55.3
0	45	98.2	3.48	2.38-5.09	
1-4	51	92.4	3.31	2.29-4.78	
5-9	63	28.6	1.01	0.71-1.43	
≥10	65	28.1	1.00		
Trimester of first prenatal visit					54.9
None	46	94.0	3.02	2.13-4.29	
First	98	31.6	1.00		
Second	64	44.2	1.41	1.03-1.93	
Third	16	33.8	1.05	0.62-1.78	

*Unknowns redistributed according to proportion of those known.
†Pregnancy-related deaths per 100,000 live births.

tension (10.3%), and cardiac arrest (10.3%) (Table II). Nonobstetric infection (6.7%) and complications of preexisting cardiovascular disease (4.0%) were the most common indirect causes. Only 18 deaths were attributed to other causes, the most common of which were homicide (3.1%) and suicide (2.2%).

Risk factors for all deaths are listed in Table III. The lowest risk of a pregnancy-related death was for women who were white and non-Hispanic, 20 to 24 years of age, married, educated beyond high school, privately insured, and primiparous, and who had received medical evaluation on more than five prenatal visits. The highest risk of pregnancy-related death was among women who were black, 40 years of age or older, unmarried, educated for 9 to 11 years, had neither private insurance nor Medicaid, and who had had five or more previous live births with little or no prenatal care.

Mortality risks were lowest for women who received prenatal care in the first trimester of pregnancy and highest for women who received no prenatal care. The

differences between groups of women who received care in the first, second, and third trimesters were not statistically significant.

Comment

The average pregnancy-related mortality ratio for New York City from 1980 to 1984 appears to be much higher than the average ratio for the United States during the same period. However, this comparison must be made cautiously, since several previous studies have shown that pregnancy-related mortality has been widely underreported in the United States. Also, the various definitions of pregnancy-related mortality used in different parts of the United States prevent true comparisons between states or regions.

Differences in populations also need to be acknowledged. The social and demographic characteristics of New York City differ from those of much the United States population. On the other hand, New York City characteristics that include an immigrant population, poverty, and drug abuse prevail elsewhere and make our findings relevant to other large urban areas. Whereas racial disparity appears highest in New York City, the pattern of excessive risk of pregnancy-related death for black women is national in scope.[26]

Ectopic pregnancy complications, embolism, and hypertension were found to be the three most frequent causes of pregnancy-related death in New York City. Kaunitz et al.[16] found similar results in analyzing 2475 pregnancy-related deaths that occurred in the United States from 1974 to 1978. However, the fourth leading cause of death in their study was not cardiac arrest, as our study found, but hemorrhage.

Whereas complications of anesthesia could be confirmed from medical records or autopsy reports as the cause of death for only four cases, these complications may have been underreported. On the death certificate, autopsy report, or medical record of at least seven deaths categorized as cardiopulmonary arrest, there was some reference to possible complications from anesthesia. However, the medical records for these seven cases were either unavailable or inadequate and thus could not confirm these complications as the cause of death.

All but two deaths classified as intrapartum cardiac arrest occurred during cesarean section. The two remaining deaths occurred during labor, one after the administration of an intravenous narcotic and the other after spinal anesthesia. At least six of the 14 deaths categorized as induced abortion complications resulted from cardiac arrest during legal abortion and were thought to result from complications of anesthesia.[27]

Previous studies have shown that the risk of maternal death increases with age for both blacks and whites, although the reasons for increased risk are not clear.[26] The association of risk of maternal death with increasing age was also seen in our study. The risk of maternal death in New York City was noted to increase with parity, and greater parity is associated with greater maternal age. Age-specific data were not available to permit adjustment for age in our analyses.

Separate studies by Kaunitz et al.[16] and by Buehler et al.[28] have found that women older than 30 have higher risks for pregnancy-related deaths than do women younger than 30.[26] Our findings are consistent with these studies and also show that risk increases with each 5-year age grouping beyond 30 years. Further studies would be useful to determine if women older than 30 are more likely than younger women to have underlying conditions that complicate pregnancy.

In conclusion, these findings suggest that women in New York City continue to suffer pregnancy-related deaths that are preventable. Lack of prenatal care and of third-party insurance are clearly associated with higher pregnancy-related mortality. The effect of other risk factors, such as age, race, education, and parity, may possibly be lessened by increased use of medical services.

Preventing pregnancy by improving access to contraception could help reduce pregnancy-related deaths. The risk associated with grand multiparity underscores the urgency of providing these services.

Comparison with prior work suggests that the pattern of causes for pregnancy-related mortality is changing. From 1970 to 1985 the incidence of ectopic pregnancies in the United States more than tripled.[29] As a result, complications of ectopic pregnancy are now a leading cause of pregnancy-related death.[11, 16, 17]

Rising rates of cesarean section expose more women to the risks of anesthesia and surgical complications. The number of deaths from cardiopulmonary arrest during labor, delivery, or abortion in a population not usually considered at high risk for cardiac disease should raise concerns.

Active surveillance for pregnancy-related deaths should be encouraged and developed, so that a more accurate analysis of pregnancy-related mortality can be studied. Since any pregnancy-related death should be considered preventable, autopsies should be encouraged or required in all such cases to help determine specific causes of death.

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Estrogen receptors in the external anal sphincter

Knut Haadem, MD,[a] Lennart Ling, MD, PhD,[b] Mårten Fernö, PhD,[c] and Hans Graffner, MD, PhD[b]

Helsingborg, Sweden

Inasmuch as anal competence in women is reduced after the age of 50 years, it may be dependent on effects of estrogens. In this study, samples of the external anal sphincter were analyzed for the presence of estrogen receptors and were found to be present at a median concentration of 5.0 fmol per milligram of protein (range, 1.9 to 13) in women (n = 7), and 1.1 fmol per milligram of protein (range, 0 to 3.2) in men (n = 7). These findings are of interest with regard to the treatment of idiopathic anal incontinence. (Am J Obstet Gynecol 1991;164:609-10.)

Key words: Anal incontinence, estrogen, estrogen receptors, external anal sphincter muscle

Maintenance of anal continence is dependent on several factors,[1] the most important of which was the tonus and contractile activity of the external and internal anal sphincter muscles. The competence of the external anal sphincter muscle is reduced with age and considerably reduced in women after the age of 50 years. One reason for this might be a decrease in the effect on the sphincters of estrogens. Because a prerequisite for this hypothesis is the presence of estrogen receptors in the external anal sphincter muscle, the present study was undertaken to ascertain whether estrogen receptors are present in this muscle.

Material and methods

The series comprised 14 patients, seven men and seven women (six of whom were postmenopausal). They all underwent abdominoperineal resection, 13 because of neoplasia and one because of inflammatory disease (Table I). In no case did the tumor involve the anus. A representative sample of the external anal sphincter muscle was taken immediately after surgery and stored at −70° C until laboratory analysis was performed.

A sample of the rectus muscle was taken from five men with a median age of 70 years (range, 52 to 78), six premenopausal women with a median age of 41 years (range, 37 to 48), and five postmenopausal women with a median age of 75 years (range, 66 to 75) to serve as controls (Table II). None of the controls had hormone-producing tumors, none was taking medication with hormones, and none of the female controls was pregnant.

Table I. Concentrations of estrogen receptors found in external anal sphincter muscle specimens

Patient characteristics			*Estrogen receptor value (fmol/mg protein)*
No.	*Sex*	*Age (yr)*	
1	Female	84	13.0
2	Female	67	12.0
3	Female	67	3.0
4	Female	36	2.9
5	Female	68	1.9
6	Female	69	5.0
7	Female	73	5.6
8	Male	78	0.0
9	Male	77	1.6
10	Male	76	0.0
11	Male	68	1.5
12	Male	64	0.5
13	Male	59	1.1
14	Male	49	3.2

Table II. Median concentrations of estrogen receptors found in rectus muscle specimens of controls

Sex	*No.*	*Estrogen receptor value*	
		Protein (fmol/mg)	*Range*
Male	5	1.3	0.98-1.8
Female			
Premenopausal	6	1.04	0.49-1.9
Postmenopausal	5	0.86	0.45-1.5

Tissue samples were homogenized at room temperature with an all-glass Potter Elvehjem homogenizer in buffer (Tris hydrochloride 10 mmol/L, ethylenediaminetetraacetic acid 1.5 mmol/L, disodium molybdenum oxygenate 5 mmol/L, and monothioglycerol

From the Departments of Obstetrics and Gynecology[a] and Surgery,[b] Hospital of Helsingborg, Helsingborg, and Department of Oncology,[c] University Hospital, Lund.
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Reprint requests: Knut Haadem, MD, Department of Obstetrics and Gynecology, Hospital of Helsingborg, Helsingborg, Sweden S-251 87.
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1 mmol/L, pH 7.4). After centrifugation at 20,000 *g* for 10 minutes, estrogen receptor content was measured in the supernatant with an enzyme immunoassay according to the kit instructions (Abbott Laboratories, Diagnostic Division, North Chicago, Ill.). The level of assay sensitivity was 2.0 fmol/ml, and estrogen receptor content was expressed in femtomoles per milligram of protein, determined according to Lowry et al.[2]

Informed consent was obtained from all patients.

Results

As shown in Table I, both the estrogen receptor content and the proportions of values in excess of method sensitivity were significantly greater in samples from female patients than in those from male patients (median estrogen receptor value, 5.0 fmol/mg protein, range, 1.9 to 13, and median estrogen receptor value, 1.1 fmol/mg protein, range, 0 to 3.2, respectively; $2p = 0.005$, Mann-Whitney U test). Two of the samples from postmenopausal patients had estrogen receptor values >10 fmol/mg protein, whereas the only sample from a premenopausal patient had an estrogen receptor value of 2.9 fmol/mg protein.

The concentrations of estrogen receptors in the rectus muscle are presented in Table II. No difference was found between sexes or between premenopausal and postmenopausal women. However, in females a significantly higher ($2p = 0.005$) estrogen receptor concentration was found in the external and sphincter than that in the rectus muscle. In the males, estrogen receptor concentrations in the rectus muscle were in the same low range as those of the external anal sphincter.

Comment

Anal continence is a complex function that involves a number of factors such as fecal consistency, anal sphincter strength, anorectal angle, and neurologic function.[1] Although fecal incontinence is a very troublesome condition for those affected, its incidence is low—a figure of 0.8% was reported for the Swedish population. The condition is usually a result of anal surgery, neurologic disease, or tears during delivery. However, in many patients direct association with a trauma or disorder cannot be found.[1] Therefore some sort of progressive pathophysiologic reduction of the contractile activity in the anal sphincter seems to take place in these patients. Because anal incontinence predominantly occurs among postmenopausal women in whom a physiologic decrease in anal competence is known to take place after the age of 50 years, it is postulated that the anal sphincter is subject to effects by estrogens.

Although the present results were obtained in a small series, it is clear that the anal sphincter muscle in females does contain estrogen receptors. This finding is in accord with reports from studies of the urethra, in which estrogen receptors have been found and considered important to the occurrence of urinary incontinence in women of postmenopausal age.[3] However, the significance of the present findings needs to be further investigated before its clinical impact can be determined. Such investigations are in progress.

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Prognostic factors in gestational trophoblastic tumors: A proposed new scoring system based on multivariate analysis

John R. Lurain, MD,[a] Lisa A. Casanova, MD,[a] David Scott Miller, MD,[a] and Alfred W. Rademaker, PhD[b]

Chicago, Illinois

All 391 patients treated for gestational trophoblastic tumors (invasive mole and choriocarcinoma) at the John I. Brewer Trophoblastic Disease Center of Northwestern University between 1969 (when use of combination chemotherapy for initial treatment of high-risk disease came into general use) and 1988 were evaluated. Univariate and multivariate analyses were used to determine the relative importance of prognostic factors with respect to survival. The overall cure rate was 93% (363/391): 100% for 223 patients with nonmetastatic disease and 83% for 168 patients with metastatic disease. The only patients who died had a clinicopathologic diagnosis of metastatic choriocarcinoma. In addition to presence of metastasis (83% vs 100%, $p < 0.0001$) and diagnosis of choriocarcinoma (67% vs 100%, $p < 0.0001$), number of metastases (47% if >8 vs 92% if ≤8, $p < 0.0001$), metastases to sites other than the lung or vagina (52% vs 91%, $p = 0.0002$), and previous failed chemotherapy (46% vs 84%, $p = 0.0014$) demonstrated independent significant effects on survival in patients with metastatic disease. A Brewer score, based on our multivariate analysis of survival in patients with metastatic disease, provided predictability of outcome (likelihood ratio χ^2 statistic, $\chi^2 = 49.8$) comparable to that with the World Health Organization score ($\chi^2 = 45.3$), both of which, in turn, were better predictors than either the traditional Hammond clinical classification system ($\chi^2 = 34.4$) or the International Federation of Gynecology and Obstetrics stage ($\chi^2 = 22.9$). (Am J Obstet Gynecol 1991;164:611-6.)

Key words: Gestational trophoblastic disease, trophoblastic tumors, choriocarcinoma

Hertz et al.[1] and Ross et al.[2] first emphasized that the successful treatment of gestational trophoblastic tumors was dependent on four factors: (1) duration of disease before initiation of chemotherapy, (2) elevation of the pretreatment human chorionic gonadotropin (hCG) level, (3) absence of central nervous system or hepatic metastases, and (4) proper administration of chemotherapy. Hammond et al.[3] subsequently showed that most patients with metastatic disease and one or more of these high-risk factors could be cured by the use of initial multiple-agent rather than single-agent chemotherapy. This led to the clinical classification of gestational trophoblastic tumors into three groups: nonmetastatic, low-risk metastatic, and high-risk metastatic, where "high risk" referred to those patients who were not likely to be cured by single-agent methotrexate or actinomycin D chemotherapy.

Bagshawe[4] later proposed a prognostic scoring system based on various factors that were thought to influence prognosis. In 1983 the World Health Organization (WHO)[5] adopted a modification of this scoring system based on patient's age, type of antecedent pregnancy, time interval between antecedent pregnancy and trophoblastic tumor, hCG level, paternal and maternal blood type, number and site of metastases, largest tumor mass, and previous chemotherapy. A weighted score was applied to each factor, each was presumed to act as an independent variable, and their effects were assumed to be additive. Patients with scores of ≥8 were regarded as high risk for treatment failure.

An anatomic staging system, based on data presented by Sung et al.,[6] was adopted by the International Federation of Gynecology and Obstetrics (FIGO) Cancer Committee in 1982.[7] Stage I tumor is strictly confined to the uterine corpus, stage II extends outside the uterus but is limited to the genital structures, stage III tumor extends to the lungs with or without genital tract involvement, and stage IV tumor is metastatic to any other site(s).

Because of the wide variety of classification and scoring systems now used to group patients with gestational trophoblastic tumors for treatment purposes and because the identification of high-risk criteria has been based on univariate analysis, there is no agreement as to which system is best at predicting outcome and which prognostic factors are most important. It is the purpose of this report to use both univariate and multivariate

From the John I. Brewer Trophoblastic Disease Center, Department of Obstetrics and Gynecology,[a] and the Biometry Section of the Cancer Center,[b] Northwestern University Medical School.
Received for publication February 13, 1990; revised July 20, 1990; accepted August 31, 1990.
Reprint requests: John R. Lurain, MD, Prentice Women's Hospital, 333 East Superior St., Chicago, IL 60611.
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analysis of commonly used prognostic factors and other descriptive features to determine the relative importance of these factors and to compare the present classification systems with a proposed scoring system based on the multivariate analysis.

Material and methods

Between 1969 and 1988, 391 patients completed treatment for gestational trophoblastic tumors (invasive mole and choriocarcinoma) at the John I. Brewer Trophoblastic Disease Center of Northwestern University Medical School. All patients underwent investigative studies before treatment, including physical examination, chest roentgenography, liver-spleen scan, brain scan, blood chemistry and hematology profile, and hCG determination. In recent years computed tomography of the brain, abdomen, and pelvis has replaced other studies. After these initial studies 223 patients (57%) were categorized as having nonmetastatic disease and 168 patients (43%) were determined to have metastatic gestational trophoblastic tumors. Seventy-three (43%) of these 168 patients with metastatic disease were further classified as being at high-risk on the basis of presence of one or more of the following: (1) immediate pretreatment hCG level in excess of 100,000 mIU/ml or serum β-subunit assay >40,000 mIU/ml, (2) time >4 months from antecedent pregnancy or onset of symptoms to treatment, (3) metastases to sites other than the lungs and/or vagina, (4) antecedent term gestation, and (5) prior unsuccessful chemotherapy.

All 223 patients with nonmetastatic disease and most of the 95 patients with low-risk metastatic disease were treated primarily with single-agent methotrexate 0.4 mg/kg or occasionally actinomycin D 10 to 12 μg/kg intravenously daily for 5 days per course. Treatment courses were repeated as often as toxicity permitted, usually every 14 days. Beginning in 1969 patients with high-risk metastatic gestational trophoblastic tumors were treated primarily with combination chemotherapy. Before 1981 the standard combination regimen used was MAC, consisting of methotrexate 15 mg, actinomycin D 0.5 mg, and cyclophosphamide 3 mg/kg (maximum 200 mg), all given intravenously daily for five consecutive days. Courses were usually repeated every 14 days, depending on hematologic recovery and toxicity. In 1981, the seven-drug modified Bagshawe protocol CHAMOCA, consisting of hydroxyurea, actinomycin D, methotrexate with folinic acid rescue, cyclophosphamide, vincristine (Oncovin), and doxorubicin (Adriamycin), replaced the MAC regimen for primary treatment of high-risk patients. After 1984 all high-risk patients were treated with the EMA-CO regimen, which included etoposide, high-dose methotrexate with folinic acid rescue, actinomycin D, cyclophosphamide, and vincristine. There were no significant differences in response with respect to chemotherapy regimen, although all patients treated after 1981 survived. Cerebral metastases were treated simultaneously with a single course of whole-organ irradiation of 3000 to 4000 cGy. Adjuvant surgical procedures, including hysterectomy and thoracotomy for excision of resistant foci of tumor, and/or irradiation of metastases at sites other than the brain were performed in selected patients.

Response to treatment was determined by hCG assays, change in size or disappearance of existing metastases, or appearance of new lesions. Chemotherapy was changed if the hCG level plateaued or rose after two chemotherapy courses or if toxicity precluded an adequate dose or frequency of treatment. Patients not responding to single-agent chemotherapy were most often treated with MAC. Patients who developed progressive disease on the MAC or CHAMOCA regimens were treated with other agents or combinations of agents including etoposide, bleomycin, and cisplatin.

During treatment, patients were monitored by physical examinations and hematology and chemistry profiles. Studies with prior abnormal results were repeated at appropriate intervals. Serum for hCG determination was obtained immediately before each treatment course. Radioimmunoassays for hCG were done from 1968 on and additionally for the β-subunit of hCG starting in 1972. Normal ranges for hCG with these assays were <20 and <2 mIU/ml, respectively. Complete remission was diagnosed after three consecutive weekly hCG levels were within normal range. At least two additional courses of chemotherapy were given after the first normal hCG level. After remission, hCG levels were obtained monthly for 6 months, every other month for the remainder of the first year, every 3 months during the second year, and at 6-month intervals indefinitely thereafter. Patients were advised not to become pregnant for 1 year after the completion of chemotherapy.

The clinical records maintained on all patients in the administrative office of the Brewer Trophoblastic Disease Center were reviewed to determine the patient's age, antecedent pregnancy, pretreatment hCG level, duration of disease, pathologic diagnosis if known, sites, size, and number of metastases, type of chemotherapy, including previous failed chemotherapy, and outcome with length of follow-up or date of death. Patients were also retrospectively assigned to FIGO stage and given a score according to a modification of the WHO prognostic scoring system (omitting ABO blood groups).

Statistical analysis of the univariate association between prognostic factors and survival was done with the log rank test. Multivariate analysis of survival time was by Cox regression analysis. Multivariate analyses were compared descriptively with a log likelihood ratio χ^2 statistic and 1 *df*. The χ^2 statistic was used as a mea-

sure of the prognostic ability of the model. Hazard ratios were used to compare across categories of a prognostic factor.

Results

The overall cure rate was 93% (363/391): 100% for 223 patients with nonmetastatic disease and 83% for 168 patients with metastatic disease. Univariate analysis was used to determine the relative effect of various prognostic factors on survival in the 168 patients with metastatic disease (Table I).

Patients with metastatic disease ranged in age from 14 to 53 years with a median age of 25 years. Fifteen patients (8.9%) were ≥40 years old. There was no effect of age on survival. The survival rate was 83% for patients <40 years old compared with 87% for patients ≥40 years old.

Pretreatment hCG values ranged from 5 to 10,000,000 mIU/ml with a median of 5000 and a mean of 18,750. Forty-eight patients (28.6%) had hCG levels >100,000 mIU/ml at the initiation of treatment. In univariate analysis hCG level had a significant effect on survival. Survival was 87% for patients with hCG values <100,000 versus 75% for patients with hCG levels ≥100,000 ($p = 0.05$).

Similarly, duration of disease had a significant effect on survival. Time from antecedent pregnancy to treatment ranged from 0 to 136 months with a mean of 6.5 months and a median of 2 months. Twenty-five percent of patients had a duration of disease >4 months. The survival rate was 90% for patients with a short duration of disease versus 64% for patients who were treated >4 months from the antecedent pregnancy ($p = 0.0003$).

The types of pregnancies associated with the development of metastatic trophoblastic tumors were: hydatidiform mole in 110 (65.5%), abortion in 29 (17.3%), ectopic in 1 (0.6%), and term in 28 (16.7%). Antecedent pregnancy had a very significant effect on survival in patients with metastatic disease ($p = 0.001$). The survival rate was 91% in patients with a preceding hydatidiform mole compared with 69% in patients with a nonmolar gestation.

Metastasis had a profound effect on survival. All 223 patients with nonmetastatic disease survived. If metastatic disease was confined to the lung and/or vagina, the survival rate was 91% (123/135) versus 52% (17/33) in patients with metastases at other sites ($p < 0.0001$). Similarly, if more than one metastatic site was involved, survival decreased from 91% (121/133) to 54% (19/35) ($p < 0.0001$). As the number of individual metastases increased, the survival rate decreased from 96% (87/91) for one to four metastases, to 84% (38/45) for five to eight metastases, to 47% (15/32) for more than eight metastases ($p < 0.0001$).

Size of the largest tumor (including uterine tumor) had a significant effect on survival in patients with metastatic disease. Tumor size was <3 cm in 114 patients, 3 to 5 cm in 33 patients, and >5 cm in 21 patients. The survival rate was 92% for patients with tumors <3 cm, versus 73% for patients with tumors 3 to 5 cm, versus 52% for patients with tumors >5 cm ($p < 0.0001$).

A clinicopathologic diagnosis of metastatic choriocarcinoma was made in 86 patients on the basis of histopathologic examination of a sufficient number of tissue specimens showing hyperplastic and dysplastic trophoblast without formed stromal villi or development of trophoblastic tumor in association with a nonmolar pregnancy. Invasive mole was diagnosed histologically in 20 patients, whereas no pathologic diagnosis could be made in 62 patients who were treated without a histologic diagnosis after evacuation of a hydatidiform mole because of plateaued or rising hCG levels with or without demonstration of metastases. All patients who died had a clinicopathologic diagnosis of metastatic choriocarcinoma. The survival rate for patients with metastatic choriocarcinoma was 67% (58/86) versus

Table I. Univariate analysis of prognostic factors with respect to survival in patients with metastatic gestational trophoblastic tumors ($N = 168$)

Variable	*No. of patients*	*% Survival*	*p Value*
Age			
≤39 yr	153	83.0	NS
≥40 yr	15	86.7	
hCG			
<100,000 mIU/ml	120	86.7	0.05
≥100,000 mIU/ml	48	75.0	
Duration			
≤4 mo	126	89.7	0.0003
>4 mo	42	64.3	
Antecedent pregnancy			
Mole	110	90.9	
Abortion	29	72.4	0.001
Ectopic	1	0	
Term	28	67.9	
Metastatic site			
Lung-vagina	135	91.1	<0.0001
Other	33	51.5	
No. of metastases			
1-4	91	95.6	
5-8	45	84.4	<0.0001
>8	32	46.9	
No. of metastatic sites			
1	133	91.0	
2	29	55.2	<0.0001
3	4	50.0	
4	2	50.0	
Tumor size			
<3 cm	114	92.1	
3-5 cm	33	72.7	<0.0001
>5 cm	21	52.4	
Clinicopathologic classification			
Choriocarcinoma	86	67.4	0.0001
Other	82	100.0	
Previous chemotherapy			
No	157	86.0	0.0005
Yes	11	45.5	

Table II. Multivariate analysis of survival in patients with metastatic gestational trophoblastic tumors ($N = 168$)*

Variable†	*Hazard ratio‡*	*p Value*
No. of metastases (1-4, 5-8, >8)	3.0	<0.0001
Metastatic site other than lung-vagina	5.3	0.0002
Previous failed chemotherapy	4.3	0.0014

*Analysis results did not change if done in 86 patients with metastatic choriocarcinoma or in 73 patients with traditional high-risk metastatic disease treated with multiagent chemotherapy, including or excluding patients who had received previous chemotherapy.

†Clinicopathologic diagnosis of choriocarcinoma excluded from model because all patients who died had metastatic choriocarcinoma.

‡Estimated increase in risk of death per unit change in variable category.

100% for 82 patients with presumed metastatic invasive mole ($p < 0.0001$).

Eleven patients with metastatic disease had received chemotherapy before referral to our center for further therapy. Only 5 (46%) of these patients were cured compared with 135 of 157 (86%) patients who were treated primarily at our center ($p = 0.0005$).

When multivariate analysis was carried out for survival, both presence of metastases and clinicopathologic diagnosis of choriocarcinoma were excluded from analysis, because only patients with metastatic choriocarcinoma died. Multivariate analysis was performed for the 168 patients with metastatic disease, the 86 patients with metastatic choriocarcinoma, and the 73 patients with traditional high-risk metastatic disease treated with multiagent chemotherapy. Number of metastases, metastases to sites other than the lung and/or vagina, and previous failed chemotherapy demonstrated independent significant effects on survival in all groups analyzed (Table II).

A comparison of the FIGO staging system, the WHO score, the traditional Hammond clinical classification, and our multivariate analysis in predicting outcome was carried out. All patients with FIGO stage I trophoblastic tumors (223) were cured. The only patient with stage II disease who died (1/10) had a large pelvic tumor complicated by hemorrhage and anemia; the WHO score was 8 and the hCG level was normal at death. Thirteen (10%) of 133 patients with stage III disease died: one with a WHO score of 6 who relapsed with brain metastases and 12 with scores of ≥8 (mean = 11). Fourteen (56%) of the 25 patients with stage IV disease died, all but two of whom had WHO scores of ≥11 (mean = 14). Two patients who died, with scores of 7 and 9, had brain and gastrointestinal metastases, respectively. There was a significant difference in survival rates between stage IV (44%) and stages II and III (90%) ($p < 0.0001$).

According to the traditional nonmetastatic and metastatic low-risk and high-risk disease classifications, 168 patients with metastatic disease were categorized into low-risk ($n = 95$) and high-risk ($n = 73$) groups. Ninety-three patients with low-risk metastatic disease (98%) and 47 high-risk patients (64%) achieved complete remission ($p < 0.0005$). The only two deaths in the low-risk group occurred in patients with WHO scores of ≥8. Among the 73 high-risk patients, 21 of 23 (91%) with a WHO score of <8 attained remission compared with 26 of 50 (52%) with scores of ≥8 ($p = 0.003$).

Modified WHO prognostic scores for the 168 patients with metastatic disease ranged from 1 to 20 with a mean of 6.3. Survival rates were 100% for 79 patients with scores ≤4, 94% for 33 patients with scores 5 to 7, and 54% for 56 patients with scores ≥8 ($p < 0.0001$).

These three classification systems were compared with a simplified prognostic scoring system (Brewer score) that is based on our multivariate analysis in metastatic cases by use of the likelihood ratio χ^2 statistic. The Brewer score was derived by assigning component scores from 0 to 3 on the basis of weights of the three significant variables from the multivariate analysis of survival. Points were assigned as follows: 0 for lung and/or vaginal metastases and no prior chemotherapy, 1 for one to four metastases, metastases other than lung and/or vaginal metastases, and prior chemotherapy, 2 for five to eight metastases, and 3 for more than eight metastases. The total score was the sum of the components. Survival in the 168 patients with metastatic disease with respect to this Brewer score was 100% (79/79) with a score of 1, 88% (37/42) with a score of 2, 63% (19/30) with a score of 3, and 29% (5/17) with a score of 4. The Brewer score had the largest χ^2 (49.8), indicating that it was the strongest of the predictors of survival outcome as compared with the WHO score ($\chi^2 = 45.3$), the traditional Hammond clinical classification system ($\chi^2 = 34.4$), and the FIGO stage ($\chi^2 = 22.9$). All four classification or scoring systems provided significant predictability of outcome ($p < 0.0001$).

Comment

After the initial reports of success of chemotherapy in the treatment of gestational trophoblastic tumors, it became apparent that certain patients with metastatic disease were more likely to fail therapy. By 1965, investigators at the National Cancer Institute had described several prognostic factors that identified a

group of patients with metastatic tumors who were likely to develop resistance to single-agent chemotherapy.[1,2] Hammond et al.[3] later showed that most of these patients with high-risk metastatic disease could be cured with initial multiple-agent chemotherapy. Additional prognostic factors were later proposed by Bagshawe[4] and Sung et al.[6] and are the basis of the WHO prognostic scoring system[5] and the FIGO staging system,[7] respectively.

The overall cure rate in the treatment of gestational trophoblastic tumors now exceeds 90%. In this study 363 of 391 patients (93%) were cured, including all 223 patients with nonmetastatic disease and 140 of 168 patients (83%) with metastatic disease. Virtually the only patients who die have high-risk metastatic choriocarcinoma when they are initially seen. Intensive therapy with combination chemotherapy and, where indicated, adjuvant radiotherapy and surgery has resulted in cure rates of 80% to 90% in this group of patients. On the basis of our analysis the prognostic factors that are most important in determining response to treatment in these high-risk patients are: (1) clinicopathologic diagnosis of choriocarcinoma, (2) metastases to sites other than the lung or vagina, (3) number of metastases, (4) previous failed chemotherapy, and (5) WHO prognostic score of ≥8 or Brewer score of 3 or 4.

The only patients who have died of trophoblastic tumor at the Brewer Center have had either histologically verified metastatic choriocarcinoma and/or tumor developing in association with a nonmolar gestation.[9-10] Of 391 patients treated between 1969 and 1988, all 82 patients with a clinicopathologic diagnosis of metastatic invasive mole survived, whereas survival in the 86 patients with metastatic choriocarcinoma was 67% ($p < 0.0001$). Goldstein,[11] Soper et al.,[12] and Azab et al.[13] have also reported a significantly poorer prognosis in patients with a clinicopathologic diagnosis of choriocarcinoma.

The site of metastasis is known to have a profound effect on survival.[1,2,4,8,13-16] At the Brewer Center, when metastatic disease was confined to the lung and/or vagina, survival was 91% (123/135) versus 52% (17/33) when other metastatic disease was present at initiation of treatment ($p < 0.0001$). Approximately half (8/15) of the patients with brain metastases and two thirds (6/9) of the patients who presented to the center with liver and/or intraperitoneal metastases died.

Additionally the number of metastases significantly affects survival. Bagshawe[4] first noted this as an important prognostic factor and incorporated this into the scoring system that was subsequently adopted by the WHO.[5] Survival decreased from 96% for patients with one to four metastases, to 84% for patients with five to eight metastases, to 47% for patients with more than eight metastases ($p < 0.0001$). Number of metastases ($p < 0.0001$) and metastatic site ($p = 0.0002$) were the two most significant factors affecting survival on multivariate analysis.

Number of metastatic sites was an important prognostic factor in univariate analysis. Survival was 91% in 133 patients with metastases to only one anatomic site compared with 54% in 35 patients with more than one site of metastatic involvement ($p < 0.0001$). In multivariate analysis, however, number of metastatic sites did not quite achieve statistical significance to be entered into the model. Soper et al.[12] and Azab et al.[13] reported a significantly decreased survival with metastatic involvement at more than one site, and the latter group found that initial presentation with metastases to more than one organ significantly affected survival on multivariate analysis (relative risk = 7.4, $p < 0.01$).

Secondary treatment of patients with gestational trophoblastic tumors yields poor results.[2,3,8,11,13-17] We previously reported that the cure rate was only 30% in 27 high-risk patients who were transferred to the Brewer Center after failure of treatment elsewhere, as compared with 63% for 46 high-risk patients treated initially with MAC at the center between 1968 and 1982 ($p < 0.01$).[14] In this report only 5 of 11 patients (46%) who had failed prior chemotherapy for metastatic disease were cured, compared with 135 of 157 patients (86%) with metastatic disease who were treated primarily at our center ($p = 0.0005$). In both this report and that of Azab et al.[13] prior unsuccessful chemotherapy exhibited a significant independent adverse effect on survival in multivariate analysis.

Other traditional high-risk factors such as hCG level, duration of disease, and antecedent pregnancy have less effect on response to treatment in high-risk patients treated with initial, appropriate multiagent chemotherapy.[4,12-17] Although these factors were significantly associated with survival in univariate analysis in our series, they did not enter into the multivariate model for survival. These factors, however, seem to have an additive effect with other, more important prognostic variables. At the Brewer Center, if one or two high-risk factors were present, survival was 74% compared with 27% in patients with three or more high-risk factors ($p < 0.005$).[14] Bagshawe,[4] Soper et al.,[12] and DuBeshter et al.[17] also observed an additive effect of these factors.

The WHO score seems to be a very important predictor of treatment outcome.[4,5,16-19] Of 28 patients who died of trophoblastic tumors at the Brewer Center between 1969 and 1988, 93% had a WHO score ≥8 (high-risk group). The average score was 13 (range 6 to 20). Ninety-one percent of traditional high-risk patients with scores of <8 were cured, compared with 52% of patients with scores of ≥8 ($p < 0.003$). The Brewer

score derived from our multivariate analysis, as outlined in this report, was also a strong predictor of survival outcome in patients with metastatic disease ($p < 0.0001$). All patients with a score of 1 were cured, compared with 88% of patients with a score of 2, 63% of patients with a score of 3, and 29% of patients with a score of 4. The simpler Brewer score ($\chi^2 = 49.8$) was comparable to the WHO score ($\chi^2 = 45.3$) in predicting survival outcome.

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Transient postpartum diabetes insipidus

A. Raziel, MD,[a] T. Rosenberg, MD,[b] P. Schreyer, MD,[a] E. Caspi, MD,[a] and Y. Gilboa, MD[b]
Tel Aviv, Israel

Diabetes insipidus may first present before, during, or immediately after pregnancy. The characteristics of two unusual cases of transient postpartum diabetes insipidus are described and discussed. (AM J OBSTET GYNECOL 1991;164:616-8.)

Key words: Diabetes insipidus in pregnancy, diabetes insipidus in puerperium, transient diabetes insipidus

From the Departments of Obstetrics and Gynecology[a] and Endocrinology,[b] Assaf Harofeh Medical Center, affiliated with Sackler School of Medicine, Tel Aviv University.
Received for publication August 7, 1990; accepted August 15, 1990.
Reprint requests: A. Raziel, MD, Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, Zerifin 70300 Israel.
6/1/24742

The coexistence of diabetes insipidus with pregnancy appears to be rare.[1] Diabetes insipidus that occurs before or during pregnancy is frequently influenced by gestation and persists for years post partum.[2] Transient diabetes insipidus is a variant of disease that occurs in pregnancy and resolves spontaneously after delivery;

Table I. Fluid intake and output and osmolality of urine and blood of the first patient with transient diabetes insipidus

Postoperative day	*Treatment*	*Fluid intake (ml/day)*	*Fluid output (ml/day)*	*Urine osmolality (mOsm/kg)*	*Blood osmolality (mOsm/kg)*	*Sodium (mEq/L)*
1	—	6200	10600	42	321	159
2	—	8820	6405	169	314	160
3	Vasopressin test*	—	3085	77	300	150
	dDAVP 100 μl × 2	—				
4	dDAVP 100 μl × 2	—	400	470	284	140
5	dDAVP 50 μl × 2	—	200	400	285	140
6	—	—	700	140	295	149
7	—	—	1250	490	280	137
8	—	—	1200	—	290	140

dDAVP, 1-Deamino-8-D-arginine vasopressin.
*Prominent response to subcutaneous injection of 5 IU vasopressin in oil.

it was formerly called diabetes insipidus gravidarum.[3] Pregnancy serves as a provocation test that unmasks possible latent diabetes insipidus; thus, the existence of diabetes insipidus that first appears after a normal delivery is highly unusual.

We describe two rare cases of postpartum transient diabetes insipidus that occurred in the *immediate puerperium only*, after non-traumatic cesarean sections with rapid amelioration and no recurrences in other pregnancies.

Case reports

Case 1. A 23-year-old primigravida woman complained of polyuria and polydipsia on the first day after cesarean section, which was performed with the patient under spinal block with tetracaine 12 mg, at 42 weeks' gestation, because of cephalopelvic disproportion. No decrease in blood pressure was noted. Intramuscular erythromycin was administered as prophylaxis. In the past she had no significant illness, and the recent course of pregnancy was uneventful. On the first postoperative day a marked polyuria and polydipsia was noted with a urine volume of approximately 11 L/day and fluid intake of 6.2 L/day. The results of plasma biochemistry tests were sodium level 159 mEq/L, hematocrit 40%, creatinine level 1.4 mg/dl, blood osmolality 321 mOsm/kg, and urine osmolality 42 mOsm/kg. In addition to fluids, large amounts of 5% glucose were administered to prevent dehydration and hypernatremia. Intravenous administration of 1 U vasopressin induced a strong antidiuresis; therefore on the third postoperative day nasal administration of two doses of 100 μl of 1-deamino-8-D-arginine vasopressin was started and reduced the high urine volume. Urine osmolality rose to 470 mOsm/kg, and blood osmolality returned to normal, 285 mOsm/kg. The prominent response to 1-deamino-8-D-arginine vasopressin is detailed in Table I.

In a search for the cause of postpartum diabetes insipidus, chest x-ray film, skull computed tomography, and visual fields were performed and found to be normal. The woman left the hospital with no complaints of polyuria and polydipsia on the tenth postoperative day and without any treatment. Three months after delivery there were no complaints and a good urinary concentration after overnight fast. One year and three years after this episode of transient postpartum diabetes insipidus, the woman underwent repeated cesarean sections in our hospital, at term while she was under epidural anesthesia with no evidence of water metabolism disturbances.

Case 2. A 36-year-old woman, gravida 3, para 2, noticed intense thirst 8 days after cesarean section. All three pregnancies had been achieved after induction of ovulation with human menopausal gonadotropins. The last two pregnancies were complicated by White's class B diabetes mellitus. A bleeding placenta previa, at 20 weeks' gestation, was the indication for hysterotomy of the first pregnancy. The second and third pregnancies ended at term by low-segment transverse cesarean sections. The last cesarean section was performed with the patient under epidural anesthesia with 2% lidocaine. Blood pressure between 120/70 and 130/70 mm Hg was measured several times during the operation. The week after the operation was uneventful. On the eighth postoperative day, a spiking fever of 38.6° C that lasted for only 24 hours was followed by polyuria and polydipsia. Fluid intake of about 4 L/day and urine volume that reached 6.2 L/day were noted, and there was no response to 5.0 IU vasopressin given subcutaneously and to gradual increase of nasal 1-deamino-8-D-arginine vasopressin up to 100 μl three times a day. On the eighteenth day of hospitalization, in view of the lack of effect of 1-deamino-8-D-arginine vasopressin, the medication was stopped. Frequent water deprivation tests were performed during the 3 months after the operation, while the patient was receiving no treatment. A gradual return to normal diuresis and drinking was observed, with similar normal changes in the osmolality of blood and urine as shown in Table II.

Table II. Fluid intake and output and osmolality of urine and blood of the second patient with transient diabetes insipidus

Postoperative day	*Treatment*	*Fluid intake (ml/day)*	*Fluid output (ml/day)*	*Urine osmolality (mOsmol/kg)*	*Blood osmolality (mOsm/kg)*
8	—	4600	6200	—	—
9	—	3600	4750	—	—
10	dDAVP 50μl × 2	2550	3850	230-280*	280-284*
11	dDAVP 50μl × 2	2880	3550	—	—
12	dDAVP 100μl × 2	3950	3950	—	—
13	dDAVP 100μl × 2	2100	3400	268-300*	291-292*
18	dDAVP 100μl × 3	2250	2400	400	285
28	—	—	1320	455-550*	290*
42	—	—	—	524	289
90	—	—	960	775*	285-286*

dDAVP, 1-Deamino-8-D-arginine vasopressin.
*Overnight water-deprivation test.

Comment

The exact incidence of diabetes insipidus appears to be two to four cases in 100,000 pregnancies, which is similar to the prevalence of diabetes insipidus in nonpregnant patients. The connection between pregnancy and diabetes insipidus is divided into three main categories: (1) pregnancy in patients with preexisting diabetes insipidus, (2) diabetes insipidus that occurs during pregnancy (including the transient form, "diabetes insipidus gravidarum"), and (3) diabetes insipidus that occurs immediately after delivery.

Pregnancy serves as a physiologic provocation test that unmasks latent diabetes insipidus. This may explain the very rare first occurrence of diabetes insipidus in the puerperium.

Among diabetes insipidus that first occurs during pregnancy there is a transient form that ameliorates after delivery. Three transient types, central type (antidiuretic hormone-responsive), nephrogenic type (antidiuretic hormone-resistant), and responsive type (antidiuretic hormone-resistant 1-deamino-8-D-arginine vasopressin-responsive), have been reported.[2]

Our two cases represent a transient form of diabetes insipidus that occurs after delivery by cesarean section; one patient was sensitive to 1-deamino-8-D-arginine vasopressin and the other was insensitive to 1-deamino-8-D-arginine vasopressin and vasopressin. The first patient returned to normal very soon after a good response to 1-deamino-8-D-arginine vasopressin that was administered nasally, and the second patient, although unresponsive to any form of antidiuretic hormone, also returned to normal during a period of 3 months.

During pregnancy there is a gradual increase in the metabolic clearance of arginine vasopressin and in placental vasopressinase activity. Levels of vasopressinase decrease at a logarithmic rate of 25%/day and become undetectable a month after delivery. Delayed decrease in vasopressinase, exaggerated metabolic clearance of arginine vasopressin, or any kind of resistance of the kidney to arginine vasopressin may cause this transient form of diabetes insipidus.

In both cases marked polyuria and polydipsia developed after cesarean section with no anesthetic complications. There was no indication that diabetes insipidus was a complication of anesthesia because both women had two cesarean sections, one of them after and the other before the occurrence of transient diabetes insipidus, and neither woman had a recurrence of diabetes insipidus.

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Postterm infants: Too big or too small?

Frances H. McLean, BScN, Mark E. Boyd, MD, Robert H. Usher, MD, and Michael S. Kramer, MD*

Montreal, Quebec, Canada

Concern over the postterm pregnancy has shifted from that of the difficult delivery of an excessively large fetus to the current concern with death in utero of an undernourished, small-for-date fetus. Studies of postterm pregnancy before the availability of ultrasonography may have included a large proportion of erroneous menstrual dates. The present study of 7000 infants was undertaken to reassess fetal growth in postterm pregnancies in which the expected date of confinement from last normal menstrual period dating was confirmed (±7 days) by early ultrasonography. Results show a gradual shift toward higher birth weight and greater crown-heel length and head circumference between 273 and 300 days of gestational age. No evidence of postterm weight loss or lower weight for length could be demonstrated. Concern in postterm pregnancy should be for fetal macrosomia, not for intrauterine growth retardation. (AM J OBSTET GYNECOL 1991;164:619-24.)

Key words: Fetal growth, postterm

The controversy as to whether postterm infants tend to be "too big" or "too small" remains unresolved. In 1902, Ballantyne described the postterm infant as excessive in size and the essential problem with delivery as cephalopelvic disproportion.[1] There have since been several studies that reported increased rates of macrosomic infants in pregnancies that progressed past term.[2-6] In contrast, Clifford[7] described the long, thin postterm infant with "the picture of recent weight loss" that he attributed to placental dysfunction, which led to fetal wasting, hypoxia, meconium release, and death. Since this description, there have been additional reports of this "placental dysfunction syndrome." The current concern of the postterm pregnancy usually relates more to fetal jeopardy from deprivation of oxygen and nutrition than to difficult delivery.

The conflicting opinions about the size of the postterm infant, the need for increased surveillance before labor, and the induction of labor have been discussed in the literature but with differing conclusions.[8,9] There appears to be no doubt that the "placental dysfunction syndrome" can occur, but there is no evidence that it occurs only or even more frequently in postterm pregnancies than it does at or before term.

One problem involves determining which infants are actually postterm. Previous studies considered infants to be postterm on the basis of the expected date of confinement calculated from the mother's last normal menstrual period. It has recently been shown that in only a small minority of infants considered postterm by last normal menstrual period could the menstrual age be confirmed by early ultrasonography.[10,11]

Another possibility is that the postterm population may include both types of infants (some too big and others too small or too scrawny); if so, population distribution by birth weight must be analyzed and not determined from mean values.

The present analysis of fetal growth characteristics and body proportions was therefore undertaken on a population in whom gestational age from last normal menstrual period was confirmed to within 7 days by early ultrasonography. Confirmed "postterm" infants (≥42 completed weeks) are compared with those born at 39, 40, and 41 weeks from the onset of the last normal menstrual period.

Population and methods

The infants studied were delivered between Jan. 1, 1978, and March 31, 1986, at the Royal Victoria Hospital, Montreal, and were previously reported with respect to obstetric management and fetal outcome.[10,12] Criteria for inclusion in the study were known date of onset of the last normal menstrual period, early (usually 16 to 18 weeks) ultrasonography measurement from which an estimated date of confinement was entered into the McGill Obstetric and Neonatal Database,[13] concordance (±7 days) of the estimated date of confinement on the basis of menstrual history and early ultrasonography, and delivery at or after 39 weeks (273 days) from onset of the last normal menstrual period.

From the Departments of Obstetrics and Gynecology, Pediatrics, and Epidemiology and Biostatistics, McGill University Faculty of Medicine, and the Royal Victoria Hospital.
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Reprint requests: Frances H. McLean, Neonatal Unit, Women's Pavilion, Royal Victoria Hospital, 687 Pine Ave. W., Montreal, Quebec, Canada H3A 1A1.
**Dr. Kramer is a senior investigator of the Fonds de la Recherche en Santé du Québec.*
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Table I. Birth weight by gestational age

Gestational age		*Primiparous women*		*Multiparous women*	
Completed weeks	*Days*	*No. of infants*	*Birth weight (gm, mean ± SD)*	*No. of infants*	*Birth weight (gm, mean ± SD)*
39	273-279	1279	3354 ± 405	1661	3474 ± 406
40	280-286	1208	3498 ± 409	1233	3608 ± 414
41	287-293	781	3604 ± 414	539	3743 ± 453
42	294-300	198	3711 ± 435	101	3859 ± 426
F_L		150.60		99.26	
p Value		<0.0001		<0.0001	

Of the 7663 infants who met these criteria, 7005 were included in this study after the exclusion of pregnancies with conditions known to disturb fetal growth (multiple births, maternal hypertension or diabetes, fetal Rh erythroblastosis, hydrops fetalis, major malformations, and TORCH [toxoplasmosis, rubella, cytomegalovirus, herpes] infections).

Infants were weighed to the nearest 5 gm on a balance scale in the nursery within an hour after delivery. Placentas with cord and membranes attached were weighed immediately after birth in the delivery room on similar scales.

Crown-heel length and head circumference were measured during the routine admission examination. These measurements have been recorded in the McGill Obstetric and Neonatal Database since 1981, and are therefore available for only 4020 of the infants.

Weight-for-length (as a measure of nutritional status) was assessed by ponderal index (birth weight in grams multiplied by 100 and divided by the cube of length in centimeters). A ponderal index of 2.2 was considered to be the lower limit of normal because this level was the 3rd percentile in a series of consecutively born infants at ≥37 weeks, as reported by Miller and Hassanein.[14]

"Severe intrauterine growth retardation" was defined as a birth weight at least 25% below the mean weight for gestational age as calculated from the infants in this study population. As indicated in a prevoius report, this definition corresponds to the 1.7th percentile for our hospital population.[15]

Growth and body proportions were compared for infants delivered within four completed 1-week periods from 39 to 42 6/7 weeks. Only five infants were delivered at 43 weeks or later; this number was too small to be included in the analysis.

The data for each week were analyzed separately for primiparous women and multiparous women, because the impaired growth has been described to occur predominantly in primiparous patients,[7] because postterm pregnancies in our population occur more frequently in primiparous women, and because infants born to primparous patients are smaller than those of multiparous patients.[10]

Statistical analyses were conducted with Statistical Analysis Systems methods.[16] Linear trends in means were tested (F test for linear trend, F_L) with the general linear models procedure. Analyses for proportions were based on Mantel-Haenszel χ^2 tests for linear trend (χ^2_L).

Results

In the infants of both primparous and multiparous women, mean birth weight increased from 39 through 42 completed weeks (Table I). Infants born to multiparous women had a consistently higher mean weight than did those born to primiparous women. A similar increase with advancing gestational age was seen for mean crown-heel length and head circumference (Table II). Infants whose length and head circumference measurements were available had mean birth weights at each gestational age that were almost identical to the birth weights of infants in the group as a whole.

The distribution of birth weights in 500 gm intervals shows a pronounced shift toward higher birth weight categories with advancing gestational age in the infants of both primiparous and multiparous women (Fig. 1). Considering all infants in the study, without regard to parity, there were no low-birth-weight (<2500 gm) infants by 42 weeks, but there was a marked increase in large (≥4000 gm) infants (9% at 39 weeks, 15% at 40 weeks, 22% at 41 weeks, and 30% at 42 weeks). Macrosomia was particularly a problem among the postterm infants (42 weeks) of multiparous women, 43% of whose infants weighed at least 4000 gm.

To determine if there was a tendency toward a bimodal distribution of birth weights (some infants were growth-retarded and others were excessively large), the distribution by 100 gm intervals was also analyzed for infants born at 39 to 40 weeks (273 to 286 days) as compared with those born at 41 weeks (287 to 293 days) and at 42 weeks (294 to 300 days). The distributions had virtually identical shapes; no evidence of bimodality was found after term (Fig. 2). Coupled with the

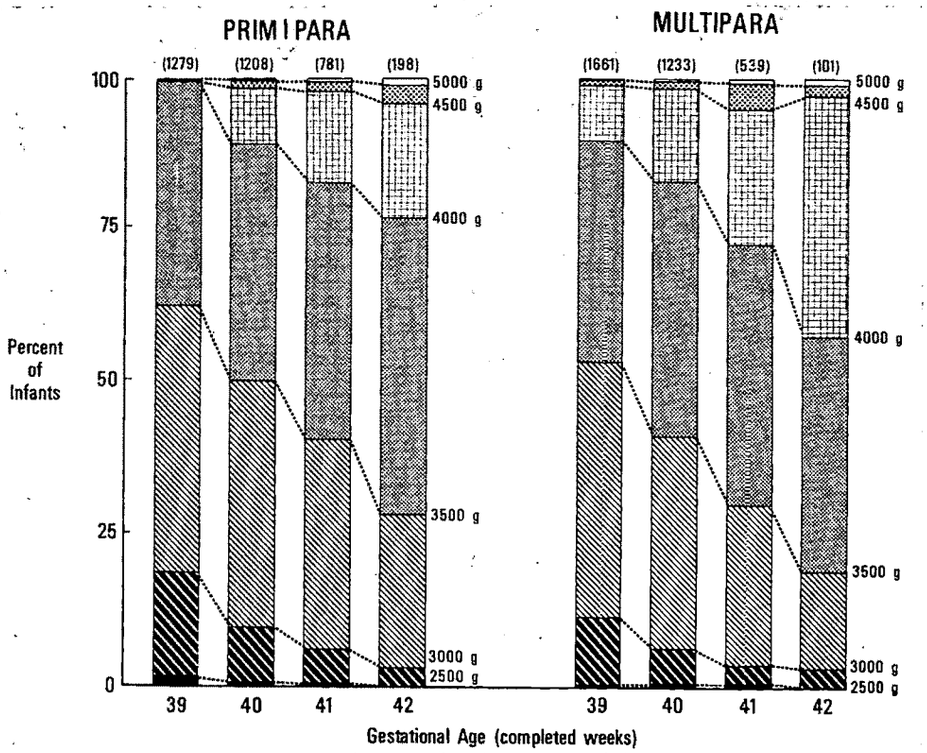


Fig. 1. Distribution of birth weight in 500 gm intervals by week of gestation.

Table II. Length and head circumference by gestational age

Gestational age		*No. of infants*	*Birth weight (gm, mean ± SD)*	*Length (cm, mean ± SD)*	*Head circumference*
Completed weeks	*Days*				
Primiparous women					
39	273-279	721	3367 ± 397	50.6 ± 2.05	34.6 ± 1.28
40	280-286	691	3497 ± 403	51.2 ± 2.16	34.9 ± 1.18
41	287-293	445	3625 ± 395	51.7 ± 2.05	35.3 ± 1.16
42	294-300	101	3735 ± 396	51.9 ± 2.47	35.5 ± 1.10
F_L			90.53	37.75	57.22
p Value			<0.0001	<0.0001	<0.0001
Multiparous women					
39	273-279	970	3491 ± 406	50.9 ± 2.24	34.9 ± 1.44
40	280-286	716	3621 ± 394	51.4 ± 2.27	35.1 ± 1.37
41	287-293	316	3744 ± 460	51.9 ± 2.28	35.4 ± 1.13
42	294-300	60	3859 ± 423	52.4 ± 2.56	35.7 ± 1.31
F_L			54.60	31.52	26.77
p Value			<0.0001	<0.0001	<0.0001

fact that the coefficient of variation (standard deviation/mean) for birth weight does not increase as gestational age progresses (Table I), these data suggest no tendency for some infants to lose weight while others continue to grow after term.

There was no tendency in either primparous women or multiparous women for severe intrauterine growth retardation to occur more frequently after term than at term (Table III). Only two of the 84 infants with severe intrauterine growth retardation were delivered after term (42 weeks).

Ponderal index at different gestational ages was also analyzed to determine if there was any evidence of intrauterine weight loss after term. None could be demonstrated, inasmuch as the mean ponderal index and the proportion of infants with low ponderal index show

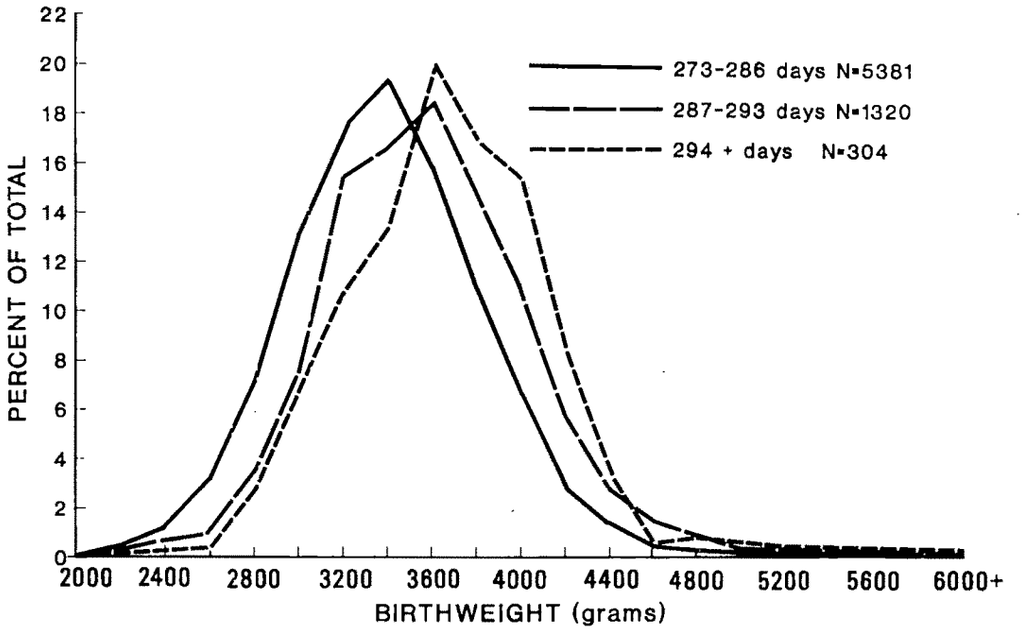


Fig. 2. Distribution of birth weight by 100 gm intervals for infants of different gestational ages.

Table III. Prevalence of severe intrauterine growth retardation*

Gestational age		*Primiparous women*			*Multiparous women*		
			IUGR			*IUGR*	
Completed weeks	*Days*	*No. of infants*	*No.*	*%*	*No. of infants*	*No.*	*%*
39	273-279	1279	23	1.80	1661	18	1.08
40	280-286	1208	11	0.91	1233	13	1.05
41	287-293	781	11	1.41	539	6	1.11
42	294-300	198	1	0.51	101	1	0.99
χ^2			1.885			0.000	
p Value			0.170			0.983	

IUGR, Intrauterine growth retardation.
*Birth weight more than 25% below mean for gestational age.

no tendency toward decreasing weight-for-length as pregnancy progressed after term; in fact, for the infants of primiparous women, the data indicate just the opposite (Table IV).

Placental weight increased as pregnancy progressed, as did infants' birth weight. Ratios of fetal weight to placental weight were constant at all gestational ages studied (Table V). There was no evidence of absolute or relative reduction in placental weight after term.

Comment

In the early years of this century, apprehension with respect to postterm pregnancy was related to the difficulty of delivery in cases of an excessively large fetus. More recently, this concern has been replaced by concern for a growth-retarded, undernourished fetus at risk of morbidity or mortality from hypoxia; this has led to an emphasis on close surveillance of postterm gestations for fetal size and well-being. However, there is a lack of convincing evidence that the postterm fetus is at increased risk of distress or nutritional deprivation. It has been the purpose of this study to determine how frequently either macrosomia (≥4000 gm) or intrauterine growth retardation develops in infants who remain in utero after term, when the "postterm" status by last normal menstrual period has been confirmed by a more reliable method.

Gestational age has been customarily calculated from the last normal menstrual period. The potential sources of error in this method are well known, although the magnitude and direction of these errors have only more recently been described.[11, 17] As measured by early ul-

Table IV. Ponderal index*

Gestational age		*Primiparous women*			*Multiparous women*		
Completed weeks	*Days*	*No. of infants*	*Ponderal index (mean ± SD)*	*Ponderal index <2.2 (%)*	*No. of infants*	*Ponderal index (mean ± SD)*	*Ponderal index <2.2 (%)*
39	273-279	721	260 ± 0.37	8.5	970	2.66 ± 0.38	5.6
40	280-286	691	2.61 ± 0.31	6.8	716	2.68 ± 0.44	5.3
41	287-293	445	2.63 ± 0.31	5.4	316	2.69 ± 0.35	6.0
42	294-300	101	2.69 ± 0.31	5.0	60	2.70 ± 0.33	3.3
F_L or χ^2_L			6.21	4.667		0.31	0.042
p Value			0.013	0.031		0.580	0.838

*Ponderal index = Birth weight (grams) × 100/Length (centimeters)^3.

Table V. Placental weight

Gestational age		*Primiparous women*			*Multiparous women*		
Completed weeks	*Days*	*No. of infants*	*Placental weight (gm, mean ± SD)*	*Fetal-placental ratio (mean ± SD)*	*No. of infants*	*Placental weight (gm, mean ± SD)*	*Fetal-placental ratio (mean ± SD)*
39	273-279	1158	641 ± 185	5.40 ± 0.96	1518	676 ± 134	5.30 ± 1.50
40	280-286	1096	663 ± 136	5.47 ± 1.80	1127	688 ± 141	5.40 ± 0.93
41	287-293	704	668 ± 144	5.56 ± 0.92	511	691 ± 143	5.56 ± 0.90
42	294-300	173	685 ± 138	5.53 ± 0.85	92	741 ± 170	5.37 ± 0.90
F_L			18.27	2.35		19.37	2.39
p Value			<0.0001	0.125		<0.0001	0.122

Fetal-placental ratio is the ratio of fetal weight to placental weight.

trasonography, the overall validity of last normal menstrual period dating for an infant born at term by last normal menstrual period is 95%, whereas the probability that an infant considered to be born after term, as based on the mother's last normal menstrual period, actually was postterm is only 12%.[11] Studies of postterm pregnancies with last normal menstrual period alone used for determining gestational age include many pregnancies with erroneous dates.

There is also an inconsistency in the definition of "postterm." Different authors consider a postterm infant to be one delivered at or after 41 weeks[6, 8] or, more frequently, at 42 weeks[3, 9, 10] or even at 43 weeks.[2, 4, 7] This issue is further confused by varying interpretations of terminology. Deliveries assigned to a certain week may have occurred during that week or after that week has been completed, or they may be of uncertain date. Gestational age in completed weeks in the present study is unambiguously defined by stating the days included.

The publication that led to the concept that infants may become undernourished if they remain in utero after term was Clifford's description[7] of the "postmaturity syndrome," which is based on 37 infants selected by clinical features who were delivered from 3 to 44 days after term. The relative risk of development of these features after term was not studied, nor was a comparison of birth weights made with controls.

After this study, Sjostedt et al.[18] classified 1171 infants as normal or abnormal on the basis of clinical inspection of the infant according to Clifford's classification but without prior knowledge of the duration of pregnancy. The mean weight of infants born between 39 and 44 weeks with signs of "postmaturity syndrome" was 200 gm less than the mean weight of "normal" infants of the same gestational age. Since the syndrome appeared to be present in some infants delivered before term, Sjostedt et al. renamed it "dysmaturity." Infants with and without signs of dysmaturity showed a similar weekly increase in birth weight from 39 to 43 weeks, but dysmature infants weighed less than the normal infants did at each week. It was concluded that although dysmaturity may be diagnosed after term, it is not caused uniquely by postterm delivery, since it can also be found before term. During the intervening 30 years since the publications by Clifford and Sjostedt et al., no published evidence was found that infants remaining in utero after term tend to become undernourished, although this concept has continued to receive wide acceptance.

However, reports of macrosomia in postterm pregnancies continue to appear.[1-6] The most recent such report was a study of 317 pregnancies with last normal menstrual period date confirmed by ultrasonography before 20 weeks. These patients were delivered of infants at 41 or 42 completed weeks.[6] Mean birth weight,

standard deviation, and frequency of infant birth weight >4000 gm in that study were similar to those found in the study cited here. Population distribution by birth weight and body measurements (and therefore ratios) of weight-for-length were not reported in this, only other study in which postdatism was confirmed by early ultrasonography.

Characteristic of all published fetal growth curves, including that from this institution,[19] is the decreased rate of growth starting about 36 or 37 weeks and a growth plateau at about 41 weeks that is followed by a subsequent decrease in mean birth weight. These curves are all based on menstrual history alone and are therefore distorted by errors in gestational age assignment in postterm deliveries.

The effect of such errors is demonstrable from the patient population in the present cohort that were excluded from analysis because early ultrasonographic dating assignments did not concur with the last normal menstrual period. The mean birth weight of all infants in this study whose mothers' menstrual dates were confirmed by early ultrasonography was 3482 gm if the infant was delivered at term (39 or 40 weeks) and 3735 gm if the infant was delivered after term (≥42 weeks). In contrast, the mean birth weight of all infants delivered after term according to a last normal menstrual period that could not be confirmed (early ultrasonography date >14 days different than last normal menstrual period date) was 3490 gm, which is very close to that of term infants in the study. Because a large majority of postterm dates are inaccurate, the overall mean in previous studies must have been markedly depressed by the inclusion of infants without confirmed dates.

Induction of labor around term, if selectively performed for growth-retarded infants, may have produced an artifactually large postterm birth weight. When the birth weight of the 18% of infants born after induction of labor between 273 and 286 days was analyzed, however, it failed to show that the induced labors produced smaller infants. Mean values within this gestational interval were 3506 gm among the infants delivered after induction of labor, as compared with 3470 gm after spontaneous labor.

When erroneous menstrual dates are eliminated with the use of early ultrasonography, mothers who are delivered of their infants after term produce large (not small) babies. Both fetal growth and placental growth continue after term. There is no evidence that infants experience growth failure or reduction in weight-for-length when pregnancy continues after term with any greater frequency than do infants delivered at term. Macrosomia, not intrauterine growth retardation, appears to be the valid concern in postterm pregnancy.

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The biologic and social consequences of perinatal cocaine use in an inner-city population: Results of an anonymous cross-sectional study

Sandra McCalla, MD,[a] Howard L. Minkoff, MD,[a] Joseph Feldman, PhD,[b] Isaac Delke, MD,[a] Martin Salwin, MD,[c] Gloria Valencia, MD,[d] and Leonard Glass, MD[d]

Brooklyn, New York

Cocaine use among pregnant women and reports of its adverse perinatal consequences have increased substantially over the past 10 years. However, most researchers have studied patients registered at drug treatment centers or have relied on voluntary participation by patients, either of which introduces the possibility of selection bias. To determine the frequency and consequences of prenatal cocaine use among an unselected inner-city obstetric population, we collected urine samples from parturient women at a municipal hospital and anonymously tested these specimens for metabolites of cocaine, marijuana, opiates, and methadone. Urine specimens, with linked obstetric data sheets, were available from a study population of 1111 patients, and pediatric data sheets were available for 846 mother-infant pairs. Cocaine metabolites were found in 11.5% of the urine samples collected, whereas metabolites of marijuana, opiates, and methadone, respectively, were present in 1.1%, 1.2%, and 0.3% of the specimens. Cocaine users were more likely than nonusers to have had no prenatal care (51% vs 8.8%; $p < 0.0001$), to be American-born rather than Caribbean-born (71% vs 33%; $p < 0.001$), and to have a higher parity (1.83 vs 1.14; $p < 0.0001$). Infants of cocaine users had a lower mean gestational age (−0.93 weeks; $p < 0.01$), a lower mean birth weight (2560 ± 788 vs 3151 ± 699 gm; $p < 0.001$), and an increased probability of having an Apgar score of <7 at 5 minutes (12.5% vs 3.2%; $p < 0.0001$). Multiple linear regression analysis that isolated confounding variables such as the presence or absence of prenatal care, maternal age and parity, and the use of cigarettes and alcohol did not substantially affect the differences described above. The effect of cigarette smoking on reducing fetal size was cumulative. In conclusion, cocaine is the most commonly used illicit drug among parturients in this community and is strongly associated with underutilization of prenatal care services. Infants of cocaine users are more likely to be preterm and depressed at birth and to have a low birth weight. Cocaine use, through the above-noted effects, increases the need for prenatal care while simultaneously decreasing the likelihood that it will be obtained. (AM J OBSTET GYNECOL 1991;164:625-30.)

Key words: Cocaine, prenatal care, birth weight, prematurity

The use of cocaine has increased markedly among young adults over the past 10 years.[1] This increase has been most notable in inner-city communities, where it has also been frequently reported among pregnant women.[2] The adverse effects of perinatal cocaine use have been widely described and include increases in low birth weight and prematurity rates and smaller head circumferences among exposed neonates.[3-6]

Most reports on cocaine use in pregnancy either have limited their study populations to women attending drug treatment centers or have relied on voluntary participation by study subjects.[3,6] The latter approach may be subject to ascertainment bias, which makes it difficult to assess the actual extent of drug use in a community. In addition, this approach may mask important social consequences of drug use, since unregistered patients would be excluded from analyses.

In an attempt to accurately define the scope of the problem of prenatal cocaine use in an inner-city community, to develop a profile of the pregnant cocaine user, and to study the consequences of recent drug use on perinatal outcome, the following study was undertaken.

From the Departments of Obstetrics and Gynecology,[a] Preventive Medicine,[b] Pathology,[c] and Pediatrics[d] at The State University of New York Health Science Center.

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Reprint requests: Sandra McCalla, MD, Department of Obstetrics and Gynecology, Box 24-450 Clarkson Ave., Brooklyn, NY 11203-2098.

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Material and methods

Thirteen hundred thirty-one women who were delivered of an infant at a municipal hospital in New York City between Oct. 18, 1988, and March 1, 1989, were eligible for entry into this study. Entry criteria were admission and/or delivery on a shift staffed

by study personnel. Specimens obtained for routine urinalysis from these parturient women were saved and given code numbers. All samples were collected within 24 hours of admission to the labor and delivery unit. They were then refrigerated and were transported within 48 hours to the institution's toxicology laboratory. Twenty patients who were delivered of their infants on these shifts were not included because they could not void while study personnel were available to collect urine.

Information that is routinely collected from parturients was recorded on data sheets, which were given the same code numbers as those of the respective urine specimens. No patient identifiers remained on data sheets, and drug screening results were never linked to patient names.

Data collected included demographic information (ethnic background, socioeconomic status, etc.); history of drug use (cocaine, marijuana, heroin, alcohol, tobacco); and pregnancy outcome measures (birth weight, gestational age as determined by last menstrual period and ultrasonogram when available, Apgar scores, mode of delivery, abruptio placentae, meconium, fetal distress).

Patients who were discharged before the research staff were able to collect the study data (for example, those who gave birth before staff off days) were not included in data analyses ($n = 200$), which left the 1111 evaluable patients that constituted the study population.

During several shifts per week, study pediatricians assessed all newborns. Pediatric assessment included head circumference, length, and gestational age based on physical examination. Data on these children were given a code number that was matched to the data for respective mothers. Pediatricians' assessments were available on 846 of the study patients. As per routine hospital protocol, urine specimens were collected for toxicologic testing on infants whom the pediatricians suspected had been exposed to a drug of abuse in utero, on either clinical or historical grounds. Two hundred twenty-seven infants, whose mothers' urine specimens had been tested, were tested for drug exposure (unpublished data). The results of these findings are the subject of a separate report. In summary, demographic and obstetric data and information on drug use were available on 1111 patients. Matched pediatric assessments were available on 846 newborns. Since gestational age based on obstetric history in a population with large numbers of unregistered patients might be somewhat unreliable, analysis with the use of gestational age was restricted to the subset from whom gestational age based on pediatric assessment was available ($n = 846$).

Laboratory techniques

Toxicology. Urine screening for illicit drugs was performed with a homogeneous enzyme-multiplied immunoassay technique (Syva Emit d.a.u., Syva Co., Palo Alto) for cannabinoid, cocaine, and opiate assays. Testing was done on a model 705 analyzer (Hitachi Co.) at room temperature (20° to 25° C). This technique has been described elsewhere[7-14]; details are available on request.

All Emit-positive test specimens were retested for confirmation by gas chromatography–mass spectroscopy. Urine samples from mothers were regularly tested the day of accession. Late specimens were refrigerated overnight and were tested the next morning. Positive specimens were stored frozen at −20° C.

Statistical methods. The study design was cross-sectional and focused on the association between the prevalence of cocaine use and various outcome measures. Continuous outcome data such as birth weight or gestational age were analyzed by multiple regression models. Multiple logistic regression models were used when outcome variables were dichotomized, for example, with an Apgar score <7. Multivariate linear models were constructed to examine the impact of cocaine on several outcomes simultaneously. In these models statistical significance was assessed by Wilk's lambda statistic. Canonical correlations were estimated to measure the strength of these associations. Contingency tables were analyzed by Fisher's test, and permutation distribution of the test statistic was used to obtain the exact probability for a table of any size on the basis of an algorithm in StatXact (Cytel, Cambridge, Mass.).

Results

The prevalence of parturients with cocaine metabolites in the urine was 11.5% (128 of 1111). Metabolites of marijuana, methadone, and other opiates were found in 1.2%, 0.3%, and 1.1% of all patients, respectively. The presence of metabolites of methadone and other opiates was detected in a similar percentage of cocaine users and nonusers. Marijuana metabolites were found more frequently in urine samples that contained cocaine metabolites (0.92% vs 3.1%; $p = 0.053$), although this trend did not reach statistical significance. Cocaine users admitted to cigarette smoking during pregnancy significantly more frequently than did nonusers (73% vs 14.3%; $p < 0.001$).

The age distribution of cocaine users was not significantly different from that of nonusers; both groups were clustered in the 21 to 30 age group (Table I). However, the ethnic distribution of the two groups was markedly different. Sixty-six percent of cocaine users were American-born blacks and 14% were from the Caribbean. Only 28% of cocaine nonusers were American-born blacks ($p < 0.001$). The mean parity of cocaine users was 1.83 (±1.65), as compared with 1.14 (±1.31) among nonusers ($p < 0.0001$).

Fifty-one percent of cocaine users had had no prenatal care, as compared with 8.81% of the nonusers.

An additional 29% of cocaine users had inadequate prenatal care (fewer than four visits to any provider) or undocumented prenatal care, as compared with 12% of nonusers ($p < 0.001$).

Drug history was found to be an unreliable predictor of recent drug use; only 54 of 128 patients (42%) with cocaine metabolites in the urine admitted to recent drug intake, which was defined as use within 1 week of admission to the hospital. Fifty-five percent of the same group acknowledged drug use at some time in the more remote past.

The frequency of low-birth-weight infants (those who weighed <2500 gm at birth) was 40.6% among cocaine users and 12% among nonusers ($p < 0.0001$). The differences in mean birth weight were also significant (2560 ± 778 vs 3151 ± 699 gm; $p < 0.001$). Significant differences were also seen in the incidence of bleeding at the time of admission (4.8% among cocaine users vs 1.5% among nonusers; $p = 0.03$) (Table II). There was a higher percentage of abruptio placentae among cocaine users, but this was not statistically significant (1.59% vs 0.41%; $p = 0.2$).

Significant differences were seen in the frequency of low Apgar score (<7 at 5 minutes; 12.5% among users and 3.2% among nonusers). Table III shows the results of logistic regression analysis for cocaine use and 5-minute Apgar scores <7, adjusting for several potential confounders. Cocaine use was the strongest predictor of a low Apgar score, with exposed infants 2.7 times more likely to receive a low score ($p < 0.01$). This was unchanged even after adjusting for gestational age.

Rates of other complications did not differ significantly between cocaine users and nonusers. These complications included premature rupture of membranes (13.0 vs 7.5; $p = 0.053$), chronic hypertension (3.4 vs 5.7; $p = 0.389$), fetal distress (19.0 vs 13.3; $p = 0.148$), meconium-stained amniotic fluid (26.9 vs 20.2; $p = 0.124$), and cesarean delivery (17.3 vs 16.9; $p = 0.90$) (Table II).

Table IV shows the results of the regression analysis for birth weight both with and without gestational age as a covariable. Because gestational age based on obstetric history in this population might be somewhat unreliable, analysis with gestational age was performed for the subset of patients from whom gestational age based on pediatric assessment was available ($n = 639$). Cocaine use was significantly associated with low birth weight (−382 gm) after adjusting for age, parity, alcohol use, cigarette use, hypertension, and prenatal care. As we expected, maternal cigarette smoking, hypertension, and absence of prenatal care each significantly reduced birth weight. After adjustment for gestation, the impact of cocaine was greatly reduced (−77.7 gm), although it was still statistically significant. Cigarette smoking remained a powerful fetal growth retardant (−104.4 gm), even after adjustment for gestational age. Absence of prenatal care did not have a significant impact on birth weight after adjustment was made for gestational age ($p = 0.296$).

Table I. Maternal characteristics of recent cocaine users and nonusers

Maternal characteristic	*Users (%) (n = 128)*	*Nonusers (%) (n = 983)*	*p Value*
Age (yr)	26.7 ± 4.62	25.5 ± 6.17	0.03
Ethnicity (%)			
Black (American)	66.4	28.0	<0.0001
Black (African)	—	2.1	
Black (Caribbean)	10.2	26.8	
Black (unknown)	10.2	7.0	
Haitian	2.3	12.0	
Hispanic (U.S.)	4.7	4.8	
Hispanic (other)	3.9	9.9	
Parity (mean, %)			
0	19.5	39.3	<0.0001
1	28.1	30.2	
2	27.3	17.0	
3	13.3	6.6	
>3	11.7	6.2	
Other substance abuse (%)			
Marijuana	3.1	0.91	0.053
Methadone	0.8	0.32	0.388
Other opiates	3.3	1.01	0.102
Tobacco use	72.7	14.44	<0.0001
Prenatal care (%)			
Institution-affiliation	29.3	78.7	
None	51.1	8.8	
Inadequate care/not documented	19.6	12.3	<0.0001

Gestational age as determined by pediatricians was itself significantly reduced in cocaine users (−0.93 weeks; $p < 0.01$), as were the head circumference (−0.64 cm; $p = 0.04$) and length (−1.23 cm; $p = 0.02$) of the infants of users (Table V). The association between cocaine use and infant head circumference (−0.16; $p = 0.54$) and length (−0.47; $p = 0.24$) was no longer significant after adjustment for gestational age.

When pediatric assessments (head circumference, birth weight, length, and gestation) were examined simultaneously, independent variables that significantly reduced growth included cigarette smoking, (canonical correlation = .0.19; $p = 0.002$), hypertension (canonical correlation = 0.15; $p = 0.03$), and cocaine use (canonical correlation = 0.13; $p = 0.02$). When the trends noted above were adjusted for gestational age, they remained the same, although only cigarette smoking remained a statistically significant growth retardant (canonical correlation = 0.154, $p = 0.001$).

Among patients with cocaine metabolites in the urine, the mean infant birth weight was smaller among those mothers who admitted to drug use (2303 vs 2669 gm; $p = 0.02$). This difference remained significant after adjustment for age and parity. There were no dif-

Table II. Pregnancy complications

Complication	*Cocaine users (n = 128)*		*Nonusers (n = 983)*		*p Value*
	No.	*%*	*No.*	*%*	
5 min Apgar score <7	16	12.5	31	3.2	0.001
Bleeding	6	4.8	14	1.5	0.021
Abruptio placentae	2	1.6	4	0.4	0.200
Hypertension	4	3.4	49	5.7	0.389
Fetal distress	16	19.0	87	13.3	0.148
Meconium staining	28	26.9	161	20.2	0.124
Cesarean section	22	17.3	166	16.9	0.900
Premature rupture of membranes	16	13.0	72	7.5	0.053

Table III. Outcome: 5-minute Apgar score <7 (n = 1111*)

Variable	*Regression coefficient*	*Odds ratio*	*95% Confidence interval for odds ratio*
Constant	−3.66	—	—
Age	0.018	1.02	0.96-1.08
Parity (0 vs 1 or more)	0.32	1.38	0.66-2.91
Alcohol (yes vs no)	−0.03	.97	0.34-2.76
Cigarette smoking (yes vs no)	0.01	1.00	0.42-2.41
Prenatal care			
Yes vs no	0.87	2.39	1.04-5.48
Inadequate vs none	0.50	1.64	0.67-4.10
Cocaine (yes vs no)	1.01	2.74	1.11-6.79

*Number for whom all data points were available.

ferences between these groups in the frequency of low Apgar scores (<7 at 5 minutes), bleeding, or other complications.

Comment

We found that 11.5% of parturients in an inner-city hospital had cocaine metabolites in the urine. Furthermore, cocaine appeared to be the most commonly used illicit drug; other illicit substances such as marijuana, methadone, and opiates were found in a much smaller number of patients. We also saw a strong association between recent cocaine use, poor obstetric outcomes, high parity, and failure to utilize prenatal services.

Several investigators have recently focused on the use of cocaine in pregnancy,[5, 6, 15, 16] but few data on the prevalence of cocaine use in an unselected general obstetric population are available.[5, 16] Researchers from Boston City Hospital[5] reported on drug use among a group of urban patients but excluded patients who had not sought prenatal care. In addition, patients in the Boston City Hospital study had the option of not being screened; 8% refused to participate. Another study by Neerhof et al.[6] evaluated patients at the time of delivery but did not cite the percent of unregistered patients included in their cohort; in addition, patients in this study had consented to entry, which introduced the possibility of ascertainment bias. In a nonselected population, we found a higher prevalence of cocaine use (11.5%) than did the investigators mentioned.

Our data on perinatal outcome confirm earlier reports. Previously described effects on birth weight, gestational age, Apgar scores, and intrapartum bleeding were noted. Other authors,[6] for example, have shown an association between cocaine use and preterm delivery, low birth weights,[5, 6] and premature rupture of membranes. We found that cocaine was associated with a 382 gm decrease in birth weight but that cigarette smoking independently reduced birth weight by an additional 125 gm. A major biologic effect of cocaine appeared to be that of shortening gestational age. Cigarette smoking exerted a negative effect on birth weight in all gestational ages.

Potentially, one of the most harmful effects of cocaine use, in addition to its biologic consequences, is its social consequences. Eighty percent of recent cocaine users in our study had had either no prenatal care or inadequate prenatal care. MacGregor et al.[17] tested the hypothesis that among cocaine-using women who were delivered of infants at their institution, those who had received comprehensive prenatal care had better perinatal outcomes than did those who had received little or no prenatal care.

Our study design also allowed us to evaluate unregistered patients. They were found to be at significantly higher risk for illicit drug use than were registered patients. Indeed, lack of care was a useful marker for cocaine use, in that 43% of patients with no prenatal care had cocaine metabolites in the urine. Patient history was, in fact, no more reliable than unregistered

Table IV. Regression analyses for birth weight and gestation

Variable	*Obstetric*		*Pediatric*	
	Regression coeffficient	*p Value*	*Regression coefficient*	*p Value*
Constant	2296.9	0.00	−4703.2	0.00
Maternal age	7.0	0.11	8.1	0.02
Parity (0 vs ≥1)	−10.6	0.83	−16.1	0.47
Alcohol (yes vs no)	−109.2	0.22	−9.7	0.80
Cigarette smoking (yes vs no)	−125.0	0.04	−104.4	0.00
Hypertension (yes vs no)	−644.6	0.00	−145.0	0.00
Prenatal care				
Yes vs no	341.2	0.00	−94.6	0.15
Inadequate vs no	191.8		44.6	0.55
Cocaine (yes vs no)	−382.4	0.00	−77.7	0.03
Gestation (wk)*	—	—	−187.6	0.00
N†	926		639	
Multiple *R*	0.39		0.74	

*Gestation was used only for those patients for whom pediatric assessment was available.
†Number for whom all data points were available.

Table V. Pediatric outcomes

Variable	*Gestation*		*Head circumference*		*Length*	
	Regression coefficient	*p Value*	*Regression coefficient*	*p Value*	*Regression coefficient*	*p Value*
Constant	37.1	0.00	31.3	0.00	45.5	0.00
Maternal age	0.008	0.65	0.024	0.12	0.052	0.05
Parity (0 vs ≥1)	0.13	0.53	−0.19	0.32	0.08	0.79
Alcohol (yes vs no)	−0.26	0.48	−0.26	0.45	−0.38	0.51
Cigarette smoking (yes vs no)	−0.37	0.18	−1.06	0.00	−1.38	0.00
Hypertension (yes vs no)	−1.60	0.00	−1.73	0.00	−3.58	0.00
Prenatal care						
Yes vs no	1.03	0.00	0.86	0.00	1.38	0.00
Inadequate vs no	0.52		0.43		0.54	
Cocaine (yes vs no)	−0.93	0.00	−0.64	0.04	−1.25	0.02
N	639		635		630	
Multiple *R*	0.31		0.39		0.37	

status in predicting toxicology results. Only 41% of patients with cocaine metabolites in the urine acknowledged cocaine use within 1 week of delivery. Those who did acknowledge use, however, may represent a group of particular risk, since they were delivered of smaller infants than were cocaine users who did not acknowledge use.

We have also found a significantly higher parity among cocaine users, which is consistent with reports of Neerhof et al.[6] This finding may be related to the fact that in poor communities women addicted to cocaine (particularly "crack") are reported to barter sex for drugs,[18] which could result in unwanted pregnancy. Increased parity among drug users has potentially devastating social consequences. Children born to parents whose addiction leaves them incapable of caring for them have already overloaded the foster care system in New York City; the census of these children increased from 16,000 in 1984 to 24,000 in 1989. These figures do include an additional 10,000 children who were being cared for by relatives other than their parents.[19]

Urine toxicology (as opposed to history), which was used to determine a patient's drug use status, subjects the study to misclassification bias. Individuals who used cocaine during pregnancy but not recently enough to be detected at the time of delivery would be classified as nonusers. Only studies that evaluated the regular urine tests of patients throughout pregnancy could avoid this bias. Cross-sectional studies at term, however, have the advantage of including unregistered patients and hence provide a description of outcomes in a subpopulation that is at particular risk. In any case the misclassification described would bias the study toward the null hypothesis and would suggest even stronger associations than those noted here.

In summary, cocaine use among parturients in the

inner-city community studied has reached epidemic proportions. Previously reported effects on low birth weight, prematurity rates, and low Apgar scores have been confirmed in this report. In addition, we found a striking association between cocaine use and failure to utilize prenatal services. This finding, along with the increased parity of women using cocaine in pregnancy, portends an increasing burden for social service institutions in urban settings. It is clear that programs designed to detect and treat pregnant cocaine users will have to use innovative means of outreach before the consequences of prenatal addiction can be addressed in any effective manner.

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The value of squamous cell carcinoma antigen in patients with locally advanced cervical cancer undergoing neoadjuvant chemotherapy

Giovanni Scambia, MD, Pierluigi Benedetti Panici, MD, Gabriela Baiocchi, MD, Mariangela Amoroso, MD, Elvira Foti, MD, Stefano Greggi, MD, and Salvatore Mancuso, MD

Rome, Italy

Serum levels of squamous cell carcinoma antigen were measured in 688 samples from 119 patients with cervical cancer. Ninety-seven patients had primary tumors and 22 had recurrent disease. Serum samples were obtained before each cycle of chemotherapy, before surgery, at least 4 weeks after surgery, and at 2- to 3-month intervals during follow-up from 78 of the patients with locally advanced cervical cancer who were receiving neoadjuvant chemotherapy. Squamous cell carcinoma antigen serum levels were elevated (>2.5 ng/ml) in 71% of the patients with primary tumors and in 77% of the patients with recurrent carcinomas. The percentage of positivity increased significantly with stage ($p = 0.03$) and was higher in squamous cell tumors than in adenocarcinomas ($p < 0.001$). Pretreatment squamous cell carcinoma antigen levels were not predictive of neoadjuvant chemotherapy response; however, the serial measurement during chemotherapy showed a good correlation with clinical response. In the patients who had surgery, squamous cell carcinoma antigen positivity did not correlate to pathologic findings (lymph node status, cervical and parametrial infiltration). Disease-free survival was significantly longer in patients with squamous cell carcinoma antigen pretreatment values that were lower than 5 ng/ml, compared with patients with marker higher than 5 ng/ml ($p < 0.01$). Abnormal squamous cell carcinoma antigen serum levels preceded the clinical detection of recurrence in eight of 11 patients with a median lead time of 5 months. (Am J Obstet Gynecol 1991;164:631-6.)

Key words: Squamous cell carcinoma antigen, cervical cancer, tumor markers, gynecologic malignancies

The management of patients with cervical cancer is hindered by limitations in clinical staging, assessment of response to therapy, and early detection of tumor recurrence. The identification of sensitive tumor markers may overcome these difficulties by permitting a more timely alternative or additional therapy.

Squamous cell carcinoma antigen, a subfraction of the TA-4 antigen, appears to be the most promising of the tumor markers that have been proposed for cervical cancer.[1-9] Although squamous cell carcinoma antigen is not useful for the early diagnosis of cervical cancer,[7-9] recent data demonstrate that in patients with invasive disease, this antigen could be employed in monitoring the response to therapy[6-9] as well as in detecting recurrences.[5, 9] However, until now, only few data are available on the serial measurements of the antigen over long periods of time in a large population of patients. In particular, only a few anecdotal cases have been reported concerning squamous cell carcinoma antigen evaluations during chemotherapy.[6-8] Moreover, in spite of a previous paper by Kato et al.,[10] which suggests that TA-4 might have a prognostic significance in cervical cancer, no other studies have addressed this point. Therefore the exact role of squamous cell carcinoma antigen in the proper management of patients with cervical cancer is still to be assessed.

We report here the experience of the Department of Gynecology of the Catholic University in the measurement of squamous cell carcinoma antigen in patients with invasive cervical cancer. Our study focuses on a large group of patients with locally advanced disease who had neoadjuvant chemotherapy followed by radical surgery[11, 12] and in whom squamous cell carcinoma antigen serum levels were serially measured for a period of 5 to 48 months. The data were analyzed to highlight the following points: (1) to determine whether squamous cell carcinoma antigen is an indicator of chemosensitivity and whether serial measurements of the antigen are a useful tool in monitoring the response to chemotherapy; (2) to ascertain whether

From the Department of Gynecology, Catholic University.
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Reprint requests: Salvatore Mancuso, MD, Department of Gynecology, Catholic University, Largo A. Gemelli, 8, 00168-Roma, Italy.
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Table I. Pretreatment squamous cell carcinoma antigen serum levels according to different variables

		SCC (ng/ml)					
		>2.5		>5			
No. of serum samples tested		*No.*	*%*	*No.*	*%*	*Median*	*Range*
Normal subjects		4	6	1	1.5	<1.5	<1.5-6
Primary tumor							
Stage I	12	7	58	4	33	2.8	<1.5-24
Stage II	43	29	67	16	37	3.2	<1.5-64
Stage III	35	27	77	18	51	5.2	<1.5-34
Stage IV	7	6	86	6	86	110	2.4-280
TOTAL	97	69	71	44	45	4.5	<1.5-280
Recurrence	22	17	77	12	55	61	15-340
Histologic type							
Squamous	79	64	81	40	51	4.3	<1.5-280
Adenocarcinoma	18	5	28	4	22	2.7	<1.5-20
Grade							
1	13	9	69	6	46	2.7	<1.5-14
2	46	39	85	24	52	4.4	<1.5-151
3	38	21	55	14	37	2.5	<1.5-280

SCC, Squamous cell carcinoma antigen. At a cutoff of 2.5 ng/ml, sensitivity was 71% (69/97), specificity was 94% (62 of 66), and positive predictive value was 75% (73/97).

pretreatment squamous cell carcinoma antigen levels have a prognostic significance; (3) to clarify the role of the antigen in the detection of recurrent disease.

Patients and methods

Our study included 688 serum samples from 119 patients (median age, 51 years; range, 31 to 74 years) with invasive cervical cancer, who were admitted to our Department from December 1987 to November 1989. Twelve patients had stage I disease, 43 had stage II, 35 had stage III, and 7 had stage IV, according to the International Federation of Gynecology and Obstetrics classification. Twenty-two had a recurrent disease. Diagnosis was always confirmed by histologic examination. Histologically, these tumors were classified as squamous in 79 patients and as adenocarcinomas in 18 patients. According to the degree of histologic differentiation tumors were found to be well, moderately, and poorly differentiated in 13, 46, and 38 patients, respectively. Seventy-eight patients with locally advanced cervical carcinoma (stage IB or IIA, tumor volume >4 cm, IIB, and III) received neoadjuvant chemotherapy according to two different schedules. The first schedule (64 patients) consisted of three cycles of cisplatin (100 mg/m^2), bleomycin (15 mg), and methotrexate (300 mg/m^2).[11, 12] Fourteen patients took part in a subsequent pilot study and received a single cycle of cisplatin (200 mg/m^2)[13] and bleomycin (30 mg). Cervical tumor size was measured by the combination of gynecologic examination, colposcopy, and pelvic ultrasonography. Stability, progression, and regression of disease were defined according to World Health Organization criteria.[14] Both protocols of neoadjuvant chemotherapy and radical surgery were approved by the Investigational Review Board, and patients gave their informed consent.

Serum samples were obtained before each cycle of chemotherapy, before surgery, at least 4 weeks after surgery, and at 2-month intervals during follow-up. Venous blood samples for marker determination were separated by centrifugation and aliquots were stored at −20° C until assayed. Squamous cell carcinoma antigen assay was performed with a commercially available kit (SCC RIA kit, Abbott Laboratories Diagnostic Div., Abbott Park, Ill.). Intraassay and interassay variations were 6.2% and 8.5%, respectively. The upper normal level was taken as 2.5 ng/ml. With this cutoff value, 62 of 66 healthy female subjects had normal marker values.

Marker titers were defined as increasing or decreasing when changes were greater than 50% of the previous value. χ^2 Test and Fisher's exact test were used to evaluate the distribution of marker values according to different variables. Life-table analysis was performed with the Kaplan and Meyer method,[15] and curves were examined by means of the z test.[16]

Results

Pretreatment evaluation. Table I shows the distribution of pretreatment squamous cell carcinoma antigen levels in relation to stage, histotype, and grade of tumor differentiation. Among patients with primary tumors, squamous cell carcinoma antigen values were found to be above 2.5 or 5 ng/ml in 71% and 45% of the cases, respectively. In these patients the percentage of positivity increased significantly with stage (p =

Table II. Pretreatment squamous cell carcinoma antigen serum levels according to chemotherapy response

Clinical response	No. of cases	SCC (ng/ml)					
		Median	Range	>2.5		>5	
				No.	%	No.	%
Complete	10	6.2	1.5–28	6	60	6	60
Partial	55	4.5	1.5–64	42	76	27	49
No change/progression	13	5.5	1.5–30	11	79	7	50

SCC, Squamous cell carcinoma antigen.

Table III. Correlation between changes of squamous cell carcinoma antigen serum levels and response to neoadjuvant chemotherapy

SCC levels	No. of cases	Clinical response			
		Complete or partial	No change or progression	Concordance rate	
				No.	%
Increased	4	—	4	4/4	100
Stable	6	4	2	2/6	33
Decreased	39	35	4	35/39	90
TOTAL	49	39	10	41/49	84

SCC, Squamous cell carcinoma antigen.

0.03). Abnormal squamous cell carcinoma antigen serum values were found in 77% of patients with recurrent disease. As previously reported,[7-9] squamous cell carcinoma antigen was more frequently expressed in squamous cell tumors than in adenocarcinomas ($p < 0.001$). Squamous cell carcinoma antigen positivity was not significantly related to histologic grading.

Squamous cell carcinoma antigen during neoadjuvant chemotherapy. Among our study population, 78 patients with locally advanced inoperable cervical cancer had squamous cell carcinoma antigen levels measured before and during neoadjuvant chemotherapy. Sixty-five of these patients achieved a clinical response (10 complete and 55 partial), whereas the remaining 13 had no change or progression of disease.

As shown in Table II pretreatment squamous cell carcinoma antigen levels were not predictive of chemotherapy response. However, in the 49 patients with positive pretreatment squamous cell carcinoma antigen levels the changes of marker values that were measured 2 weeks after the end of chemotherapy with respect to pretreatment values were closely related to the clinical course of the disease (Table III). The overall correlation between the clinical course of the disease and the variation of squamous cell carcinoma antigen levels was 84% (41 of 49). Interestingly, the changes of squamous cell carcinoma antigen levels after two cycles of chemotherapy predicted the clinical outcome in 86% of cases. On the other hand, after one cycle of chemotherapy, the predictive value of the squamous cell carcinoma antigen assay was only 57% (data not shown). This low figure is due to the fact that in eight patients who achieved partial response at the end of the three cycles of treatment squamous cell carcinoma antigen levels remained stable after the first cycle, whereas in seven patients squamous cell carcinoma antigen levels initially decreased even though the final clinical evaluation showed no change of disease.

Squamous cell carcinoma antigen levels and pathologic findings at surgery. In sixty-five patients surgery was possible after neoadjuvant chemotherapy and underwent surgery that consisted of type III-IV radical hysterectomy with systematic paraaortic and pelvic lymphadenectomy. Table IV shows the distribution of squamous cell carcinoma antigen values according to some of the most important pathologic findings. It appears that squamous cell carcinoma antigen positivity measured both before and after neoadjuvant chemotherapy did not correlate with lymph node status, depth of tumor penetration, or parametrial involvement.

Squamous cell carcinoma antigen levels and prognosis of cervical cancer. Fig. 1 shows that among patients who received neoadjuvant chemotherapy the disease-free interval curve significantly differed ($p < 0.01$) according to squamous cell carcinoma antigen pretreatment values. Moreover, when pretreatment squamous cell carcinoma antigen values were analyzed in relation to recurrence of disease, it appeared that

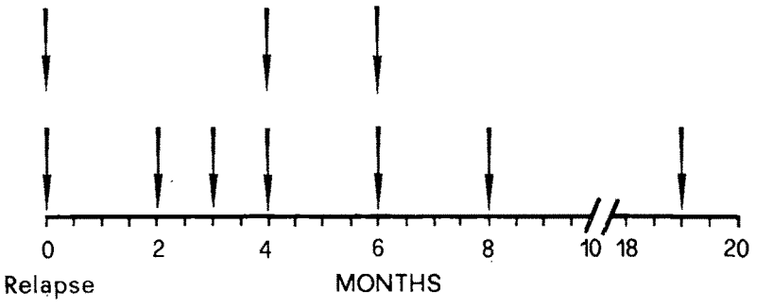


Fig. 1. Disease-free survival of patients who had surgery according to squamous cell carcinoma antigen serum levels.

Table IV. Serum levels of squamous cell carcinoma antigen according to pathologic prognostic factors

	SCC (ng/ml)												
	Before chemotherapy							*After chemotherapy*					
				>2.5		*>5*				*>2.5*		*>5*	
	No.	*Median*	*Range*	*No.*	*%*	*No.*	*%*	*Median*	*Range*	*No.*	*%*	*No.*	*%*
Lymph node status*													
N−	50	4.0	1.5-64	37	74	21	42	2.1	1.5-23	23	46	9	18
N+	15	7.2	1.5-34	12	80	9	60	1.6	1.5-16	7	47	4	27
Depth of tumor penetration													
<5 mm	19	4.3	1.5-28	15	79	9	47	2.5	1.5-5.6	10	53	2	10
>5 mm	46	4.5	1.5-64	34	74	21	46	2.6	1.5-23	23	50	10	22
Parametrial involvement†													
−	51	3.8	1.5-64	38	75	20	39	3.0	1.2-14	27	53	8	16
+	14	2.0	1.5-34	6	43	5	43	2.3	1.5-23	5	36	4	28

SCC, Squamous cell carcinoma antigen.
**N*−, *N*+; Absence and presence of lymph node metastases, respectively.
†−, +; Absence and presence of parametrial involvement, respectively.

higher antigen levels were present in patients who had recurrences than in those who did not. With a cutoff value of 5 ng/ml (Table V) recurrence rate was 73% in patients with positive squamous cell carcinoma antigen levels compared with 41% in those with negative values ($p = 0.05$). Even though the number of patients is still too small to allow final conclusions, the difference between patients with positive squamous cell carcinoma antigen and those with negative levels persisted even when patients were subdivided by stage, although it reached a statistical significance only for patients who had stage III carcinoma ($p = 0.03$).

Squamous cell carcinoma antigen and detection of recurrences. Over the median follow-up period of 25 months (range, 5 to 48 months) after surgery, 11 patients had recurrent disease. As shown in Fig. 2, elevated serum values of squamous cell carcinoma antigen preceded the clinical detection of recurrences in eight cases with a median lead time of 5 months. In two of three patients with negative pretreatment squamous cell carcinoma antigen levels, an abnormal value was detected at the time of the clinical diagnosis. In the last patient the squamous cell carcinoma antigen level never rose.

Comment

Our study demonstrates that squamous cell carcinoma antigen represents a useful biochemical diagnostic tool for the proper management of patients with invasive cervical cancer.

Pretreatment squamous cell carcinoma antigen levels did not differ among patients who responded and those who did not respond to neoadjuvant chemotherapy, which suggests that squamous cell carcinoma antigen expression is not an indicator of chemosensitivity. However, in the large series of patients with locally advanced tumors who were examined in this study, squamous cell carcinoma antigen proved to be very well correlated with the clinical response to neoadjuvant chemotherapy. Interestingly, the changes of squamous cell carcinoma antigen levels after two of the three planned cycles of chemotherapy were good predictors of the final clinical outcome. This suggests that the serial assessment of squamous cell carcinoma antigen concen-

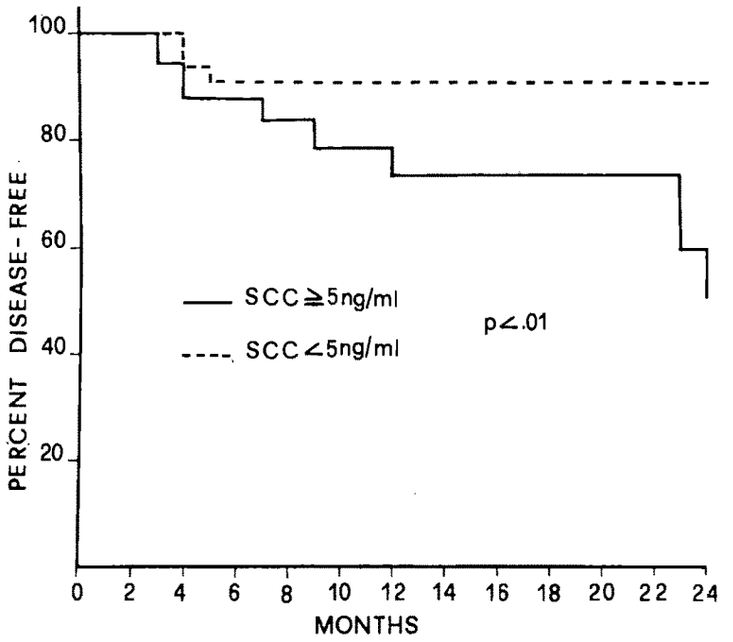


Fig. 2. Lead time between serum squamous cell carcinoma antigen rise and clinical detection of recurrence (0 time) during follow-up. *Arrows* indicate first appearance of a squamous cell carcinoma antigen value higher than 2.5 ng/ml. *SCC,* Squamous cell carcinoma antigen.

Table V. Squamous cell carcinoma antigen levels and tumor recurrence

	No. of cases	*SCC (ng/ml)*				*Median*	*Range*
		>2.5		*>5*			
		No.	*%*	*No.*	*%*		
Overall							
NED	54	38	70	22	41*	3.4	1.5-64
REC	11	8	73	8	73	14	1.5-34
Stage I-II							
NED	32	22	69	11	34	3.1	1.5-64
REC	5	2	40	2	40	6.4	1.5-14
Stage III							
NED	22	16	73	11	50†	4.8	1.5-34
REC	6	6	100	6	100	17.7	4.5-34

SCC, Squamous cell carcinoma antigen; *NED,* no evidence of disease; *REC,* recurrence.
*$p = 0.05$.
†$p = 0.03$.

trations during neoadjuvant chemotherapy may add important clinical information that would lead to timely use of alternative or additional therapies.

It is worth mentioning, however, that during the early phases of chemotherapy, misleading results can be found. The initial fall of squamous cell carcinoma antigen levels in patients who are not responding to neoadjuvant chemotherapy suggests that in some cases a tumor component may initially respond to therapy.

Squamous cell carcinoma antigen might have a prognostic role in cervical cancer. Patients with positive squamous cell carcinoma antigen serum titers have a higher recurrence rate and a reduced disease-free interval compared with patients with negative squamous cell carcinoma antigen titers. This is consistent with observations made by Kato et al.[10] and more recently by Maiman et al.[9] It remains to be demonstrated whether squamous cell carcinoma antigen measurement may represent a prognostic factor that is independent from other variables. To obtain this confirmation, work is in progress on a large patient population. However, the finding that the difference in the recurrence rate be-

tween patients with positive SCC and those with negative SCC stage III is still statistically significant seems to be encouraging. Moreover, the lack of correlation between squamous cell carcinoma antigen levels and some important pathologic prognostic factors, such as parametrial involvement, lymph node status, and depth of tumor penetration,[17] further supports this hypothesis.

Squamous cell carcinoma antigen measurement proved to be very useful in the detection of recurrent disease after surgery. In patients with high pretreatment squamous cell carcinoma antigen levels, the elevation of the antigen preceded the clinical detection of disease with a median lead time of 5 months. This confirms previous observations made by Maiman et al.[9] who reported that squamous cell carcinoma antigen preceded recurrence by an average of 5 months. Previously, Kato et al.[5] reported that elevated TA-4 levels preceded the clinical detection of disease recurrence in 11 of 17 patients.

Our study clearly shows that, at least in patients with locally advanced cervical cancer, the serial measurement of squamous cell carcinoma antigen is particularly useful in those patients with high pretreatment values. In fact, among patients with negative squamous cell carcinoma antigen values, elevation of squamous cell carcinoma antigen was detected in two cases only when disease recurred after surgery. This conclusion cannot be applied to patients with early cervical lesions. In such cases, even though tumor cells can express squamous cell carcinoma antigen, the serum antigen titers could be within the normal range because of the low tumor burden. Therefore in these cases an immunohistochemical evaluation of squamous cell carcinoma antigen expression on the tumor specimens could be more useful to select candidate patients for squamous cell carcinoma antigen monitoring during therapy and follow-up.

In conclusion, our study provides evidence that the squamous cell carcinoma antigen serum assay should be routinely assessed in patients with invasive cervical cancer. Serial measurements of the antigen seem to be useful to monitor response to therapy and might also influence clinical decisions concerning the discontinuation and change of therapy as well as the early use of second-line therapies. Further clinical trials should now verify the role of squamous cell carcinoma antigen levels as a prognostic factor of cervical cancer.

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Neonatal complications at term as related to the degree of umbilical artery acidemia

Carey L. Winkler, MD, John C. Hauth, MD, J. Martin Tucker, MD, John Owen, MD, and Cynthia G. Brumfield, MD

Birmingham, Alabama

The degree of umbilical arterial acidemia associated with immediate newborn morbidity has not been determined. Therefore we compared 358 term infants with umbilical artery acidemia (pH < 7.20) with 358 term, nonacidotic matched control infants, to evaluate immediate neonatal complications in both groups. Nonacidotic was defined as an umbilical artery pH ≥7.20. Complications included seizures, persistent hypotonia, and/or signs of end-organ damage such as renal or cardiac dysfunction. None of the 693 newborns with an umbilical artery pH ≥7.00 had such complications. Two of 23 infants with an umbilical artery pH <7.00 had sequelae related to intrapartum asphyxia. In these two infants the umbilical artery pH was <7.00, the 1-minute and 5-minute Apgar scores were ≤3 and the acidemia was metabolic in nature. (Am J Obstet Gynecol 1991;164:637-41.)

Key words: Acidemia, complications, neonatal, term

Until recently, peripartum asphyxia was commonly believed to be the primary cause of cerebral palsy, mental retardation, and other forms of neurological dysfunction. More recent studies now suggest that the causes of cerebral palsy are unknown and that intrapartum events may account for only a small fraction of such cases.[1-5] In spite of the use of continuous electronic fetal heart rate monitoring systems and an ever-increasing cesarean section rate, the incidence of cerebral palsy has remained essentially unchanged for the last two decades.[6]

Asphyxia is defined as cessation of gas exchange, with subsequent hypoxia and hypercarbia. In utero, total cessation of gas exchange is rare, but varying degrees of hypoxia and transient interference with maternal-fetal respiratory exchange are common in all labors. If significant hypoxemia occurs, the fetus will utilize anaerobic glycolysis to meet its energy needs. The subsequent formation of nonvolatile acids such as lactic acid will result in a decrease in the blood pH of the fetus. Various investigators have established normal ranges for umbilical artery pH and blood gas values.[7-12] Most have found that the mean umbilical artery pH ranges from 7.25 to 7.30, with 2 SD below the mean ranging from 7.15 to 7.20. Thorp et al.[7] have reported a large series in which normal ranges for umbilical cord blood gas levels at term were established. They determined the mean umbilical artery pH to be 7.24, with SD of 0.07 pH units. Thus in their series a pH of 7.10 was a statistically low pH, i.e., <2 SD below the mean umbilical artery pH. Ruth and Raivio[8] selected an umbilical artery pH of <7.16 as <2 SD below the mean. We have previously reported that 2 SD below the mean umbilical artery pH is 7.18.[9] However, the population from which this figure was derived was selected to have been at term gestation, to have had a spontaneous vaginal delivery, and to have had a normal fetal heart rate baseline and variability in the second stage of labor. Wible et al.[10] defined a low umbilical artery pH as <7.20. Thus, despite the traditional definition of umbilical artery acidemia as a pH <7.20,[13] most recognize that this level is arbitrarily high and may range as low as the 7.10 suggested by Thorp et al.[7] No one has demonstrated any significant biologic correlation between these statistical definitions and the subsequent immediate neonatal performance. We have surmised, as did Gilstrap et al.,[14] that at least one partial explanation for the apparent lack of correlation of umbilical artery cord gas parameters and immediate neonatal asphyctic sequelae is the utilization of the arbitrarily high values of normal and standard deviation for the umbilical artery pH. Thus the denominator has been diluted with the normal values of normal newborns. The inclusion of newborns who have a statistically significant low pH value (with the use of cutoff values as low as 7.10, 7.15, 7.18, or 7.20) has had little correlation to fetal biologic damage at term gestation, as reflected by a newborn course compatible with intrapartum asphyxia. We have hypothesized that the effect of an intrapartum period of hypoxia or asphyxia that is sufficient to cause pro-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Alabama at Birmingham.
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Reprint requests: John C. Hauth, MD, University of Alabama at Birmingham, Department of Obstetrics and Gynecology, University Station, Birmingham, AL 35294.
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tracted fetal and neonatal damage is not a linear function. Rather we have observed that only below a certain umbilical artery pH, with a metabolic component to the acidemia and with a low 5-minute Apgar score, will the incidence of newborn asphycitic complications increase.

We sought to determine the correlation between the degree of umbilical artery acidemia, type of acidemia, and Apgar scores and the immediate neonatal complications that have commonly been correlated with adverse neurologic outcomes.[15, 16]

Material and methods

This study is based on 2764 term deliveries that occurred from October 1987 to April 1989. In all of these deliveries, paried umbilical artery and vein blood gas determinations were made. A group of fetuses was selected whose gestational age was ≥37 weeks, who had paired umbilical artery and vein cord gas samples, and whose umbilical artery pH was <7.20. During this time period, 358 mother-infant pairs met these criteria, and their charts were retrospectively reviewed. The control group consisted of 358 mother-infant pairs with delivery occurring during the same time period with similar inclusion criteria, except that the umbilical artery pH was ≥7.20. Each acidotic fetus was matched with a control by obtaining the next subsequent fetus matched for mode of delivery and indication for operative delivery.

Blood samples were drawn from a doubly clamped segment of umbilical cord into a 1 ml syringe flushed with 1/1000 heparin solution and placed on ice. Blood gas determinations were performed with an ABL 330 blood gas analyzer (Radiometer, Copenhagen). Umbilical artery acidemia was classified as follows[17]: metabolic—umbilical artery pH <7.20, P_{CO_2} < 65 mm Hg, and bicarbonate ≤17 mEq/L; respiratory—umbilical artery pH <7.20, P_{CO_2} ≥65 mm Hg, and bicarbonate >17 mEq/L; mixed—umbilical artery pH <7.20, with P_{CO_2} ≥65 mm Hg and bicarbonate ≤17 mEq/L or with P_{CO_2} <65 mm Hg and bicarbonate >17 mEq/L.

Maternal records were reviewed for antenatal complications, mode of delivery, use of oxytocin, and type of anesthesia. Neonatal charts were reviewed for Apgar score, birth weight, and immediate neonatal complications known to be associated with intrapartum asphyxia. These complications included neonatal seizures, persistent hypotonia, and evidence of end-organ dysfunction such as acute cardiac or renal failure.

The data were analyzed using the SAS statistical software package (Statistical Analysis Systems, Cary, N.C.). The Student t test, χ^2, and analysis of variance were used where appropriate. Statistical significance was defined as a p value ≤0.05.

Results

There was no significant difference between the two groups as to parity, race, use of oxytocin, gestational age at delivery, birth weight, sex, operative delivery, indication for operative delivery, diagnosis of fetal distress, or neonatal complications (Table I). There were two neonatal deaths in the control group, one from lethal anomalies, and the other from group B streptococcus sepsis. One infant, a thanatophoric dwarf with secondary respiratory insufficiency, died among the group with acidosis.

Three hundred fifty-eight of 2764 term newborns had an umbilical artery pH of <7.20. The mean umbilical artery pH of the study population was 7.13 (range 6.65 to 7.19), and that of the control population was 7.26 ($p < 0.001$). Sixty-eight newborns (19.0%) had an umbilical artery pH <7.10, with 23 (6.4%) of these pH values <7.00. The average 1-minute and 5-minute Apgar scores were significantly lower in the 23 newborns with an umbilical artery pH <7.00 (Fig. 1) when compared with those 693 whose pH exceeded 7.00. The number of Apgar scores ≤3 at 1 minute was significantly greater in the acidemia group than in the control group (18 vs 7, respectively, $p = 0.025$). This difference in the 1-minute Apgar score mostly occurred as the result of including those newborns with an umbilical artery pH <7.00. At 1 minute, 7 of 23 newborns (30.4%) with an umbilical artery pH <7.00 had an Apgar score ≤3, as compared with 11 of 335 newborns (3.1%) with a pH of 7.00 to 7.19 and 7 of 358 newborns (2.0%) with a pH ≥7.20. At 5 minutes the Apgar scores for all pH groups were not significantly different.

Fifty-five (15.4%) of the study population had a metabolic acidemia, 156 (43.6%) a respiratory acidemia, and 147 (41.1%) a mixed pattern. The type of acidemia was not associated with any significant difference in the incidence of low Apgar scores at 1 or 5 minutes as compared among the group with acidemia or with the control population. Asphyctic newborn complications occurred only in the mixed group (Table II).

For the acidemia group, the distribution of the different types of acidemia (metabolic, respiratory, or mixed) were equal throughout the pH ranges. Only two infants in the acidemia group had asphyctic complications. A description of each of these cases follows.

Case reports

Case 1. After an uncomplicated prenatal course the patient presented to the antepartum clinic with severe hypertension, proteinuria, and headache. On admission to the hospital, after a 4 gm intravenous bolus of magnesium sulfate, the patient had a tonic-clonic seizure that continued in spite of further magnesium and valium therapy. Fetal bradycardia then occurred. General anesthesia was induced to control the convulsions and to permit an emergent cesarean delivery. The 3620

Table I. Demographic and intrapartum comparison data for newborns with an umbilical artery pH of <7.20 or ≥7.20

	Newborn umbilical artery pH		
	<7.20 (n = 358)	*≥7.20 (n = 358)*	*p Value*
Maternal age (yr)	24.3	23.4	0.03
Parity			
0	179	188	NS
≥1	179	170	NS
Race			
Black	252	253	NS
White	106	105	NS
Infant sex			
Male	188	188	NS
Female	170	170	NS
Birth weight (gm)	3351	3362	NS
Mode of delivery			
Spontaneous–outlet forceps	169 (47%)	170 (47%)	NS
Low forceps	69 (19%)	68 (19%)	NS
Mid forceps	13 (4%)	12 (4%)	NS
Cesarean section	107 (30%)	108 (30%)	NS
Intrapartum oxytocin	132 (37%)	145 (41%)	NS
Delivery for distress	135 (38%)	118 (33%)	NS

Table II. Type of umbilical artery acidemia compared with incidence of low Apgar scores and newborn complications

			Apgar score 0-3		*Asphyxial newborn complications*	
Type of acidemia	*No.*	*%*	*1 min*	*5 min*	*No.*	*%*
Metabolic	55	15.4	3 (5.4%)	0	0	0
Respiratory	156	43.6	6 (3.8%)	0	0	0
Mixed	147	41.1	9 (6.1%)	2 (1.3%)	2	1.4
Control pH ≥ 7.20	358	NA	7 (2.9%)	1 (.03%)	0	0

NA, Not available.

gm female infant was born at term. Apgar scores were 2 at 1 minute and 4 at 5 minutes. The umbilical cord gases showed a pH of 6.65, P_{O_2} of 2 mm Hg, P_{CO_2} of 169 mm Hg, and bicarbonate of 15 mEq/L. The infant needed oxygen for 2 days but subsequently died after heart failure from severe tricuspid valve regurgitation caused by ischemic necrosis of the papillary muscle after the neonatal period. Review by our neonatal faculty concluded that intrapartum hypoxia and asphyxia resulted in irreversible fetal cardiac damage.

Case 2. The patient was admitted at term for induction of labor because of severe oligohydramnios. During labor, mild to moderate variable fetal heart rate decelerations occurred, followed by persistent severe bradycardia. An emergency cesarean section was performed. The infant weighed 3620 gm and had an Apgar score of 1 at 1 minute and at 5 minutes. The umbilical artery cord blood showed a pH of 6.96, P_{CO_2} of 89 mm HG, P_{O_2} 8 of mm Hg, and bicarbonate of 16 mEq/L. The neonatal course was complicated by acute renal failure, major motor seizures, and a requirement for supplemental oxygen for 2 days.

Comment

The causes of cerebral palsy and other neurologic disorders remain unknown. Previously, many infants with these dysfunctions were thought to have had intrapartum asphyxia. However, it has become apparent that at most, only 10% of the incidence of cerebral palsy results from intrapartum asphyxia.[1, 2, 4, 5] In spite of numerous advances in the evaluation of the neonate in the immediate newborn period, we are still unable to predict adverse neurologic sequelae. Moreover, the degree of severity of intrapartum asphyxia-acidemia that is required to cause neurologic damage to the fetus is still unknown.

The presence of neonatal hypoxic encephalopathy (major motor seizures, persistent hypotonia) identifies those infants most likely to have long-term neurologic disorders.[16, 18] The presence of hypoxic encephalopathy, however, identifies only those with the most severe asphyxia. A marker of more subtle changes is desirable. Many authors have shown that the 1-minute and 5-minute Apgar scores are not useful as markers for as-

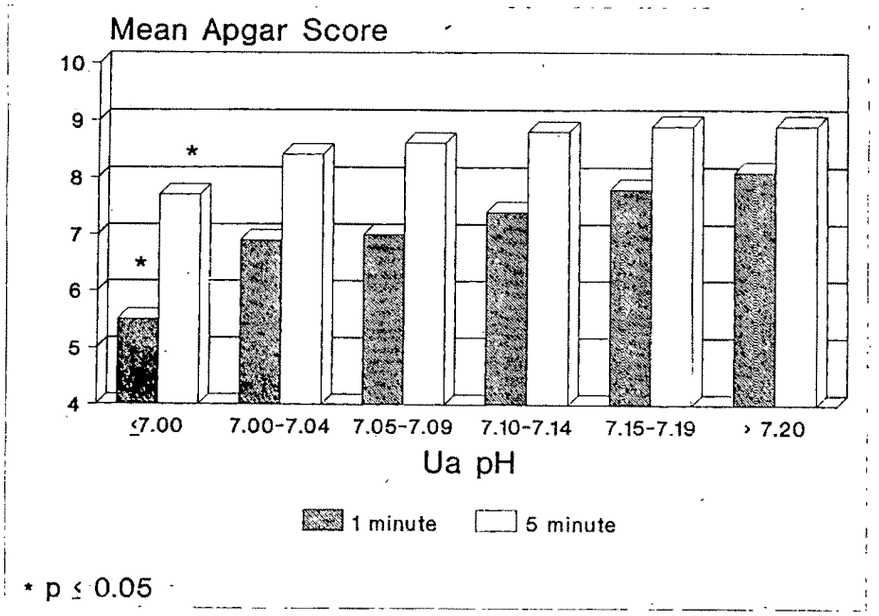


Fig. 1. Low Apgar scores as related to level of umbilical artery pH.

phyxia, since they cannot be used to accurately predict neurologic problems during infancy.[1-4, 18] Since sustained fetal hypoxia results in acidemia, many researchers have advocated using the level of umbilical artery acidemia as a measure of the severity of the intrapartum hypoxia. Neonatal acidemia is defined as an umbilical artery pH <7.15 or <7.20, because these values are 2 SD below the mean umbilical artery pH of normal infants.[13] However, few reports have correlated either the type or the degree of umbilical artery acidemia with neonatal complications. Gilstrap et al.[14] suggested that newborns are only at risk for immediate complications that result from intrapartum asphyxia when the umbilical artery pH is <7.00 and Apgar scores are ≤3 at both 1 minute and 5 minutes. However, their series evaluated only one umbilical sample. Moreover, in spite of the presence of nonacidotic neonates in the study population, neonates with acidemia were not matched to nonacidemic controls.

The purpose of our review was to ascertain which type and degree of acidemia, if any, places newborns at increased risk for immediate newborn complications. Our review was, by intention, limited to term infants, to eliminate the many confounding variables unique to preterm infants.[8, 19] Our study group was matched with nonacidotic controls to assess the background incidence of complications.

Mean 1-minute and 5-minute Apgar scores were lower when the umbilical artery pH was <7.00. However, if the pH was >7.00, there were no significant differences in Apgar scores when the study population and the control population were compared. The type of umbilical artery acidemia did not predict low Apgar scores, either at 1 minute or at 5 minutes. Asphyctic complications (persistent hypotonia, neonatal seizures, evidence of end-organ damage, or persistently low Apgar scores) occurred in only 2 of the 358 acidotic newborns and were not seen in any members of the control group. Each of the two infants who died had both a severe mixed acidemia and low Apgar scores at 1 minue and at 5 minutes. In spite of the presence of severe acidemia (pH ≤7.00), only 2 of 23 infants (9%) had significant complications. None of the remaining 66 infants with an umbilical artery pH <7.10 manifested ominous signs or symptoms in the neonatal period.

In our series we were unable to demonstrate a statistically significant difference in the incidence of short-term neonatal complications that were attributable to acidosis (2/358 vs 0/358, $p = 0.25$, Fisher's exact test). Therefore we performed a power analysis to determine the difference in acidemic complications that could have been detected in our study population. With an α level of 0.05, this study has an 80% chance of detecting a difference in morbidity of 0.36%, if such a difference actually exists.

Thus we could not confirm that infants born with an umbilical artery pH <7.20 are at greater risk for short-term neonatal complications attributable to intrapartum asphyxia than are those infants with a pH ≥7.20. However, when newborns have a severe acidemia (umbilical artery pH <7.00), especially with a metabolic component, as well as low 1-minute and 5-minute Apgar scores, they may be at increased risk for neonatal complications of the types commonly associated with

neurologic sequelae. Nevertheless the absolute risk is undetermined, since in our series of 358 term acidotic deliveries only 2 of 23 (9%) infants with severe acidemia had newborn complications indicative of intrapartum asphyxia. A larger study of severely acidotic infants would be required to define this risk more precisely.

In summary, the positive predictive value of umbilical cord gas analysis for immediate newborn complications associated with presumed intrapartum hypoxia is poor. However, umbilical artery blood gas analysis is useful in excluding intrapartum asphyxia.

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Urinary tract dilatation in pregnancy

Mark A. Brown, MD
Sydney, Australia

Right-sided ureteral and renal pelvis dilatation was observed during routine uterine ultrasonographic examination at 30 weeks' gestation. This continued to progress with the renal pelvis measuring 8.9 cm in diameter at 35 weeks, leading to early delivery. Renal function remained normal and these changes resolved completely after delivery. (Am J Obstet Gynecol 1990;164:641-3.)

Key words: Hydronephrosis, pregnancy, renal pelvis

Dilatation of the ureters and renal pelves are well-recognized accompaniments of normal pregnancy. Numerous studies have sought to determine whether this dilatation is physiologic (a result of ureteral smooth muscle relaxation) or anatomic (a result of compression of the ureter by the fetus, particularly on the right-hand side as it crosses the iliac artery at the pelvic brim) but this issue remains unresolved.[1,2] Surprisingly, in the course of these studies no data were provided to help clinicians recognize what degree of urinary tract dilatation can occur during normal pregnancy and it is therefore difficult to know when such dilatation is evidence of a disease process. We recently managed a case of massive urinary tract dilatation in pregnancy that caused us to deliver the patient early, although the changes resolved completely and spontaneously postpartum.

Case report

A 29-year-old Irish woman attended the antenatal clinic for a routine visit at 30 weeks' gestation in her fourth pregnancy. Her first pregnancy 8 years previously had been complicated by mild pregnancy-induced hypertension and she had since had two normal pregnancies. Ultrasonographic examinations were not performed in any of the previous pregnancies. She had always been in good health and was unaware of any family illnesses. She had not taken any medication throughout this pregnancy, was a nonsmoker, and did not consume alcohol. Routine uterine ultrasonography confirmed her stage of gestation and showed normal fetal anatomy. However, the proximal right ureter was noted to be dilated during this scan and was traced to the renal pelvis. The ureter measured 2 cm in diameter in its proximal portion and the right renal pelvis was 7 cm (Fig. 1). The appearance of the left urinary tract was considered normal. Blood pressure was 120/70 mm Hg, there were no specific urinary tract symptoms, results of urinalysis and urine microscopy were normal, and there was no evidence of urinary tract infection. Serum creatinine level was 0.04 mmol/L and serum electrolyte values were normal.

A repeat scan was made 3 weeks later at which time the renal pelvis had increased to 8.2 cm in diameter (Fig. 1). The patient remained without symptoms and normotensive. Serum creatinine level was 0.06 mmol/L. Two weeks later the right renal pelvis measured 8.9 cm and there was a slight decrease in renal cortical width (Fig. 1). Serum creatinine level was 0.05 mmol/L and blood pressure and urinalysis were normal. Because of the progressive enlargement of the renal pelvis and possible reduction in cortical width, she was admitted to the hospital and labor was induced at 36 weeks' gestation. She was delivered of a male infant who weighed 3175 gm and is well.

Two days postpartum renal ultrasonography showed the right renal pelvis was reduced to 2.6 cm diameter and the ureter in its proximal portion to 1 cm (Fig. 1). An intravenous pyelogram showed enlarged kidneys, 17 cm on the right and 16 cm on the left, with no evidence of urinary tract obstruction or any intrinsic ureteral lesion. Serum creatinine value was 0.05 mmol/L. A further ultrasonogram was obtained 3 months postpartum and showed that kidney size had returned to normal and the renal pelvis and ureter were completely normal.

Comment

This case shows massive dilatation of the right ureter and renal pelvis accompanying normal pregnancy. To our knowledge this degree of urinary tract dilatation has not been reported previously and therefore we considered this to be abnormal for pregnancy and more likely to reflect intrinsic ureteral disease. The fact that these changes resolved so rapidly and completely postpartum prove that they were entirely caused by pregnancy and were not a result of a ureteral lesion. In

From the Departments of Renal Medicine and Obstetrics, St. George Hospital.
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Reprint requests: Mark A. Brown, MD, 1st Floor Main Building, St. George Hospital, Kogarah, Sydney, Australia NSW 2217.
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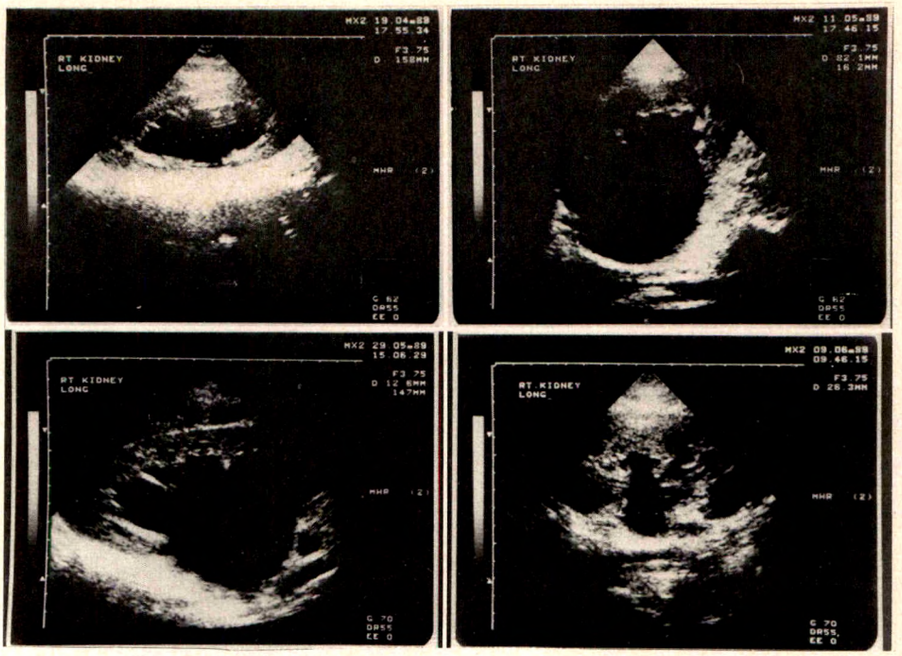


Fig. 1. Renal ultrasonographic recordings at 30 weeks' gestation *(top left)*, 33 weeks *(top right)*, 35 weeks *(bottom left)*, and 2 days postpartum *(bottom right)*. Renal pelvis enlarged from 7 cm at 30 weeks to 8.9 cm at 35 weeks.

retrospect there was no maternal indication for delivery and long-term renal function from the right kidney was unlikely to be jeopardized, so that induction of labor was probably unnecessary.

It is unknown whether such a degree of urinary tract dilatation is common during pregnancy inasmuch as no study has documented the degree of dilatation in a systematic fashion. However, on the basis of this case it seems reasonable to allow pregnancy to progress normally and delivery to occur spontaneously even in the face of such massive ureteral and renal pelvic dilatation in pregnancy, provided that the patient is without symptoms and there is no evidence of impairment of renal function.

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Clinical implications of screening for cervical cancer under Medicare

The natural history of cervical cancer in the elderly: What do we know? What do we need to know?

Jeanne Mandelblatt, MD,[a] Clyde Schechter, MD,[b] Marianne Fahs, PhD,[c] and Charlotte Muller, PhD[c]

New York, New York

Despite the recent passage of coverage for Papanicolaou test screening under Medicare, several aspects of the natural history of cervical cancer in the elderly remain uncertain. This article reviews what we know about cervical cancer in elderly women to provide clinicians with the background necessary for assessments of screening recommendations, integration of new data into practice, and development of consensus approaches to screening in the elderly. Two central questions that affect a screening program for the elderly are how long the neoplastic process takes from preinvasive disease to the development of invasive cancer, and how likely is it that a given neoplastic state observed in an elderly woman will, in fact, progress to a more severe state. The ultimate success of the new Medicare benefit will also be affected by the use of Papanicolaou testing, the technique of obtaining the smear, and the adequacy of reporting and follow-up. The expansion of Medicare benefits to include early cervical cancer detection has the potential to improve the quality and the duration of older women's lives. (Am J Obstet Gynecol 1991;164:644-51.)

Key words: Cervical cancer, elderly, Medicare, Papanicolaou smears

Contrary to popular belief, cervical cancer remains an important health problem into old age and most groups now conclude that screening needs to reach older women to be maximally effective in decreasing the morbidity and mortality from this condition.[1-5] Toward this end, Medicare coverage was extended to include triennial Papanicolaou testing beginning in July 1990.[6] Whereas passage of this benefit should solve the problem of financial barriers, several aspects of the clinical implementation of the program remain unresolved. Because uncertainties still exist in our knowledge about cervical cancer, there is no consensus approach to the screening, diagnosis, and treatment of cervical neoplasia, particularly for elderly women.

This article reviews the controversial and uncertain aspects of the natural history of cervical cancer in elderly women to provide clinicians with the background necessary for informed assessment of screening recommendations and integration of new data, as they become available, into practice. For the review, published studies from 1950 to 1988 were examined with the use of a standard abstraction form summarizing location, methods, quality of the data, and the applicability of the findings to older women.

We examined factors that may affect the usefulness of screening in the elderly, including the following: (1) how quickly the disease progresses from cervical intraepithelial neoplasia (defined as cervical intraepithelial neoplasia grade 1 or mild dysplasia and cervical intraepithelial neoplasia grade 2 or moderate dysplasia) to carcinoma in situ (defined as cervical intraepithelial neoplasia grade 3 or severe dysplasia and carcinoma

From the Memorial Sloan-Kettering Cancer Center,[a] Mount Sinai School of Medicine,[b] and Division of Health Economics, Mount Sinai School of Medicine.[c]
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Reprint requests: Jeanne Mandelblatt, MD, Memorial Sloan-Kettering Cancer Center, Division of Cancer Control, Box 60, 1275 York Ave., New York, NY 10021.
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in situ), and finally to frankly invasive carcinoma (expressed by the duration in each state); (2) what proportion of preinvasive disease develops into invasive cancer in elderly women; (3) Papanicolaou test characteristics; (4) hysterectomy status; and (5) cohort and subgroup risks.

Disease state duration

The goal of screening for cervical cancer is to diagnose and treat cervical cancer in its early, preinvasive states, such as cervical intraepithelial neoplasia and carcinoma in situ, when survival is close to 100%. If these preinvasive states have a long duration, say 10 years, then the clinician can safely screen most women every 3 to 5 years. Alternatively, if the duration of preinvasive disease is as short as 1 or 2 years then screening will need to be performed annually. Thus the disease state duration is a major determinant of the appropriate periodicity of screening.

Unfortunately, there is no consensus about the duration of cervical neoplasia in women of all age groups, and even less agreement about duration in elderly women. Four methods have been used by researchers to estimate the duration of the various states of cervical neoplasia. The first method estimates duration through direct observation of women with disease and followed up without intervention.[7] This method is now considered unethical unless the subjects have been offered treatment and have refused.

A second method of estimating the duration of each state of neoplasia uses the ages at which the largest number of cases occur (the mode or modal age) to infer the time from the start of a state to the start of the next state. For example, if the modal age of carcinoma in situ were 35 years and the modal age for invasive cancer were 50 years, the estimated average duration from the beginning of carcinoma in situ to the beginning of invasive cancer would be estimated as 15 years.

However, a few caveats should be noted that may affect conclusions about the elderly on the basis of the use of this method. First, estimates obtained as a result of subtracting modal or mean ages of incidence only yield a correct estimate if each state being considered can be measured from the identical point in its evolution. Because younger women are most likely to have lesions diagnosed through routine screening, their lesions are detected close to the actual onset of neoplastic changes. Older women, in contrast, who are not routinely targeted in screening programs, are more likely to have lesions diagnosed closer to the end of the neoplastic spectrum, when lesions are more likely to produce symptoms such as vaginal bleeding. In this situation, the use of modal or mean ages to estimate duration will overestimate the true duration from the beginning of a state, such as carcinoma in situ, to the beginning of the next state, invasive cancer, to the extent that symptomatic cases are represented in the population.

That this method does, in fact, yield overestimates is supported by the observation that carcinoma in situ duration derived in this manner is twice the duration estimated for the same population database with other methods.[8, 9]

In addition, this method may obscure cohort differences because data used are generally cross-sectional.[9] When cohort risk varies from generation to generation, prediction of disease duration from data on previous generations may not be accurate for current or future groups of elderly women. Despite these cautions, this technique has been widely used because it is simple and uses readily available data.

A third method to assess duration uses the method described by MacMahon et al.[10] in which the average duration of a disease, from onset to termination, is a ratio of prevalence to incidence in a given time period. Dunn[11] and Kashgarian and Dunn[12] draw on this relationship and concluded that older women and black women have shorter durations of carcinoma in situ than do women in general. For white women, they estimated carcinoma in situ duration to be 5 to 16 years for women under age 65 years and only 1 year for women age 65 and older. However, it should be noted that the estimates are made on the basis of the results of Papanicolaou smears obtained from samples in which women aged 20 to 39 years were overrepresented and women 55 to 74 years were underrepresented.

In addition, the accuracy of estimates with the use of incidence and prevalent cases defined by Papanicolaou smear results will be reduced by misclassification errors. Also, the estimates will be correct only if the incidence and prevalence rates are constant over the time period of measurement and if both are measured from the same state in the disease process, as noted above. If prevalence rates are increasing or if screening rates differ, the estimates can also be distorted. Although age-specific rates are incorporated into the estimates from the large-population screening program used by Kashgarian and Dunn,[12] the estimates of duration may also have a wide range of error when applied to older women as a result of small numbers of elderly being screened. Last, these estimates assume similar mortality rates for women at all ages. For elderly women, who have higher mortality rates than younger women, this assumption may underestimate the true duration of cervical neoplasia. Despite the potential limits, other researchers using similar methods and different data sets have derived comparable estimates of duration.[9, 13]

A fourth method, mathematical modeling, is used to estimate both the duration of different disease states and the proportion of lesions in each state that pro-

gresses to the next state. One such technique is Markov modeling, which simulates the progress of a population through various disease states.[14] Markov models are homogeneous when the probability of moving between states is kept constant over time, or nonhomogeneous when the transition probabilities are allowed to depend on time or age.

Richart and Barron[15-18] used a homogeneous Markov process to assess the duration of cervical intraepithelial neoplasia in two quite different data sets and derived similar results from each.[15-18] They concluded that the duration of cervical intraepithelial neoplasia was independent of age, that is, that it was constant across different age groups. It should be noted that whereas the data they used for their estimates were very reliable, the estimates were made on the basis of cervical intraepithelial neoplasia rates in women aged 20 to 39 years.

Coppleson and Brown[19] attempted to show that the results based on age-independence for carcinoma in situ did not fit the observed data. They used data collected by Bibbo et al.[20] in a nonhomogeneous (age-dependent) Markov model for their estimates. In the large series reported by Bibbo et al. 12% were over 49 years old; there is no information on the actual age distribution. Coppleson and Brown assumed these older women to be age 65 for their model. From the estimates of the duration of carcinoma in situ derived from their models they concluded that duration of cervical neoplasia in elderly women was one quarter that of younger women. However, the assumption that women over 49 years old are age 65 may invalidate their conclusions for women aged 50 years and older and probably underestimates the transition times of older women. Also, estimates obtained as a result of this type of data pooling may lack precision. Hence, whereas it may be true that an age-independent model does not fully explain the observed natural history of the disease, the Coppleson and Brown analysis does not resolve the issue.

Table I summarizes the estimates of carcinoma in situ duration from studies with both age-dependent and age-independent models. The median estimate of carcinoma in situ duration derived with age-independent models is 9.5 years for all ages, compared with the age-dependent models, which estimate 16 years for younger women, 4 years for women aged 50 years or older, and 1 year for women aged 65 years and older. Therefore the duration of carcinoma in situ in the elderly may be as long as 9 years or as short as 1 year.

The new Medicare benefit for Papanicolaou testing covers screening at 3-year intervals. The choice of this interval is influenced by the need to provide the most benefit per dollar spent for the overall population of elderly women.[21] From the clinical perspective, triennial screening should be adequate to detect most disease in preinvasive states. However, if preinvasive cervical cancer states have a shorter duration in older women, as compared with younger women, recommended screening intervals may need to be adjusted. Future cohort studies that focus on older women may provide the information needed to resolve this issue.

Probability of progression and regression of each state

To determine the most appropriate diagnostic and treatment course for an elderly woman with abnormal Papanicolaou smear findings, the clinician will also need to know the likelihood that a given lesion will progress to a more severe state. If a lesion is certain to progress to invasive carcinoma, an aggressive work-up is warranted. But, if a lesion is more likely to regress to a less severe state or remain static, conservative management could be considered.

Unfortunately, the probability of remaining in a given state, progressing to the next state, or regression to the prior state is as difficult to determine as durations, particularly for the elderly. The best estimates of disease behavior are made on the basis of groups of women with cervical intraepithelial neoplasia and carcinoma in situ who were followed up without intervention. Because it is unethical to follow up women without treating them once carcinoma in situ develops, the probability of progression from carcinoma in situ to invasive cancer is even more difficult to assess than that for progression from cervical intraepithelial neoplasia to carcinoma in situ.

On the basis of a longitudinal follow-up study of untreated cervical intraepithelial neoplasia, Barron and Richart,[17] estimated that after 10 years 66% of all cervical intraepithelial neoplasia lesions will progress to carcinoma in situ, 28% will remain in cervical intraepithelial neoplasia, and 6% will revert to normal. Regression to normal occurred only from very mild or mild cervical intraepithelial neoplasia.

In contrast, Fox[22] followed up 278 women with cervical intraepithelial neoplasia and noted that 31% of lesions regressed to normal, 9% remained in cervical intraepithelial neoplasia, and 60% progressed to carcinoma in situ. Several women regressed after termination of pregnancy or completion of antiinfection treatment, but in 13% of the women whose smears originally regressed to normal cervical intraepithelial neoplasia subsequently developed. The redevelopment of cervical intraepithelial neoplasia after regression to normal has been noted in other series.[23, 24] In fact, in one of these series, among women over age 45 years (ages 45 to 65) whose initial cervical intraepithelial neoplasia lesions regressed, 40% subsequently recurred per year.[24] All these findings suggest that misclassification biases have resulted in overestimates of the regression rate of cervical intraepithelial neoplasia.

Estimates of the probability of regression from cer-

Table I. Duration of cervical neoplasia

Study	*Year*	*Study type*	*Study population*	*Age group (yr)*	*CIS duration (yr)*	*Age relationship*	*Method of estimation*
Canadian Task Force	1976	Cross-sectional	Canadian women; included 12% of elderly population	All ages	9.7-13.4	Independent	Mean age
Fidler et al.	1968	Cross-sectional	12% of elderly women	All ages	6-9.5	Independent	Prev/incid = duration
				All ages	12	Independent	Mean age
Peterson	1956	Longitudinal	212 Danish women referred for specialty care from 1930-1950; a few over 40	All ages	3.7 (range, 0.4-8.8)	Independent	Direct observer
Barron et al.	1978	Cross-sectional	Data from women in Barbados and British Columbia	All ages	3-10	Independent	Prev/incid = duration
Coppleson, Brown	1975	Cross-sectional	Data from women attending family planning clinics, and national data from the Third National Cancer survey	<50	17	Dependent	Markov model
				≥50	4	Dependent	Markov model
Kashkargian, Dunn	1970	Cross-sectional	>110,000 women in Memphis, 2%	25-35	16	Dependent (using age-specific data)	Prev/incid = duration
				40-50	5	Dependent (using age-specific data)	Prev/incid = duration
				>65	1	Dependent (using age-specific data)	Prev/incid = duration

CIS, Carcinoma in situ; *prev*, prevalence; *incid*, incidence.

vical intraepithelial neoplasia to normal have also been derived from studies in which lesions have been diagnosed through biopsy specimens. The proportion of cervical intraepithelial neoplasia lesions observed regressing to normal in these settings ranges from 20%[25] to 47%.[26] The lower rates of regression noted in nonbiopsy specimen studies compared with the biopsy specimen studies cited above suggests that the act of establishing the diagnosis altered the results.[15]

Estimates of the regression of carcinoma in situ range between zero[7] and 25%.[22] In one series in which women with carcinoma in situ were followed up without treatment (as a result of refusing treatment or loss to follow-up), no regression was noted among women age 40 years and older, whereas 32% of carcinoma in situ cases regressed among women under age 40.[27] Coppleson and Brown,[19] with the use of a data set that included women over the age of 45 years, concluded that the probability of disease progression from carcinoma in situ to invasive cancer rises with increasing age. A lower regression rate of cervical intraepithelial neoplasia has also been observed among older women compared with younger women.[24]

Whereas these conclusions are consistent with clinical observation, it is also possible that the apparent increased likelihood of progression with increasing age is an artifact of cross-sectional data in conjunction with historically low screening rates for older women. Nonetheless, a higher probability of disease progression is biologically plausible: the elderly have been noted to have decreased immune response compared with younger populations and immunosuppressed women in a variety of settings have been noted to have high rates of cervical neoplasia[28, 29]; in addition, changes in the hormonal environment with menopause have been proposed as promoters of neoplasia.

Given the current state of our knowledge of the behavior of cervical neoplasia, most groups recommend that elderly women with abnormal Papanicolaou smear findings receive a thorough colposcopic examination and, if the transformation is not fully visualized, a cone biopsy should be performed.[21] New techniques may also become available, such as cervigrams, which may be helpful in guiding the treatment of elderly women.[30]

In addition, as the implications of the presence of human papillomavirus (HPV) in cervical neoplastic lesions is more fully understood, simultaneous Papanicolaou and HPV testing holds the promise of guiding treatment protocols. In one study of HPV and cervical intraepithelial neoplasia, Campion et al.[31] noted that 90% of cervical intraepithelial neoplasia grade 1 lesions positive for HPV type 16 progressed to cervical intraepithelial neoplasia grade 3 in an average of 22.4 months. Moreover, only 7% of cervical intraepithelial neoplasia lesions regressed and none of these contained HPV type 16. This study, based on a small, selected sample of young women in a referral colposcopy clinic, needs to be confirmed in other populations and other age groups before recommending guidelines on the basis of HPV status.

Also, there are no data yet on HPV infections in elderly women in this country. The only U.S. study of HPV in older women of which we are aware is a description of HPV in hysterectomy specimens, where surgery was performed for nonmalignant conditions.[32]

In Germany, deVilliers et al.[33] examined more than 9000 women for HPV types 16/18 and 6/11; 10% of the study sample were aged 60 years or older.[33] They found that the association between HPV and cervical intraepithelial neoplasia, and HPV and normal smears, generally decreased with age, although there is some suggestion from their data that the age-specific distribution of HPV in cervical intraepithelial neoplasia lesions is bimodal, with the second peak at about age 75 years. Of note was their finding that among women with invasive cancer, HPV was present more often in elderly women than in younger women. This observation supports the hypothesis that age-related changes may interact with the neoplastic process.

Papanicolaou test characteristics in the elderly

Accuracy of the Papanicolaou test at all ages is crucial if the smear is to perform effectively as a screening test. The accuracy of the Papanicolaou test has two basic components: the sampling component and the evaluation component. Each of these includes both avoidable errors (e.g., inadequate sampling or errors in reading a slide) and unavoidable errors (e.g., because of the biologic characteristics of the lesion) that prevent Papanicolaou smear accuracy from being 100% in any age group. In addition, several characteristics of the elderly patient may influence the accuracy of the Papanicolaou smear; these include anatomic changes associated with aging, particularly decreased accessibility of the squamocolumnar junction.

Cervical cancer arises at the junction of the squamous epithelium of the vagina and the columnar epithelium of the endocervix.[34] The area where these two meet is referred to as the squamocolumnar junction, which must be sampled for accurate Papanicolaou testing. The location of this junction varies with age. In younger women it is located at the external cervical os, moving onto the exocervix after childbearing, and then back into the endocervical canal after menopause.[35]

Movement of the squamocolumnar junction higher into the cervical canal with increasing age renders this area less accessible for sampling. In addition, failure of exfoliation of abnormal cells is more likely in postmenopausal women than in younger women.[36] Moreover, vaginal and cervical os stenosis may hinder sampling.

A study by Roberts et al.[37] shows some of these potential pitfalls in examining older women. In this series, 50 postmenopausal women with negative Papanicolaou smear results underwent colposcopy. Eleven women had positive results, four (8%) of these being cervical intraepithelial neoplasia.[37] The discrepancy between the Papanicolaou test and the colposcopy observed in this study was ascribed to diminished exfoliation of cells and anatomic inaccessibility of the transformation zone to sampling in older women. Although this was not a random sample of older women, the finding of a high rate of false-negative smears is consistent with the anatomic changes seen in this age group.

In addition to clinical sampling errors, evaluation errors may also affect the accuracy of the test. Evaluation errors may lead to false-negative results when abnormal cells are missed and to false-positive results when normal cells are identified as abnormal. In general, false-negative rates vary with age, study, and setting, and the state of the lesion, and have been reported to range from 5% to 35%,[15, 38-42] although most of these rates were obtained in populations of women whose average age was 35 years.

There is a similar paucity of primary data on the false-positive rate of cervical smears among elderly women. In general, false-positive rates described for nonelderly women have ranged from less than 1% to 5%.[21, 38, 41, 43] In two recent reports of Papanicolaou screening of elderly women false-positive rates of 0.1%[44] and 0.3%[45] occurred. The clinician should be particularly alert to atrophic smears because the normal atrophic changes that occur in postmenopausal women can result in vulnerability to inflammation and injury and these responses can mimic varying degrees of neoplasia.[44, 46, 47] Koss[47] discusses these and other pitfalls of cervical cancer screening in his recent review of Papanicolaou testing.

The recent guidelines for classification of Papani-

colaou smears recommended by the National Cancer Institute Workshop (Bethesda system)[48] addresses many of the potential problems in Papanicolaou screening for the elderly. The Bethesda system recommends that Papanicolaou smear reports comment on the adequacy of the specimen, provide a general categorization of the results as either normal or other, and contain a descriptive diagnosis. If a specimen does not contain endocervical components the smear should be repeated. The descriptive diagnoses used in the Bethesda system should allow the clinician to distinguish between infection and reactive-reparative changes and epithelial cell abnormalities. This descriptive system also incorporates a recommendation for further evaluation. Thus the cytopathologist will play an integral role in assuring that specimens from elderly women are properly obtained, interpreted, and followed up.

Other parameters

Other parameters that the clinician should consider that may influence the yield of screening programs for aged women include hysterectomy prevalence and cohort effects.

Hysterectomy status. Older women who had hysterectomies should not automatically be excluded from screening programs. Before the early 1960s many women had partial hysterectomies that left the cervix intact. In one study, one third of the elderly women with a history of a hysterectomy had an intact cervix on clinical examination.[44] Between 4% and 8% of cervical cancer arises in these cervical stumps.[49, 50] Also, women who had a hysterectomy for malignant disease have a high risk of subsequent development of vaginal cancer.[51] Therefore a careful history including the date of surgery, type of operation performed, and indications for the hysterectomy is important in determining which older women should continue to receive Papanicolaou smears. Often an initial speculum examination is also necessary to verify the patient's status. In the future, the majority of women with a history of a hysterectomy are likely to have had surgeries that removed the cervix. At that point, the reason for the prior surgery will be the best guide to the need for continued Papanicolaou surveillance.

Cohort effects. Several authors have noted variations in cohort incidence and mortality rates.[52, 53] The implications of such cohort trends for clinical practice are not clear and many questions remain with regard to current and future cohorts of women: is the current cohort of elderly women, who were generally beyond their reproductive years when Papanicolaou screening was beginning to be widely used, only at risk because of a lack of prior regular screening; will future cohorts of elderly women, who will have had more extensive screening as younger women, continue to need surveillance; will HPV infection increase risk of future groups of women; will different subgroups within age cohorts, such as poor and minority women, continue to be at higher risk than nonpoor, white women? It behooves physicians to research these issues and ensure that new data are incorporated into screening recommendations in a timely manner.

The answers to these questions also have important implications for clinical practice. Cervical cancer screening in the elderly will be most effective if providers concentrate on screening those women, who, by virtue of socioeconomic status, race, or other personal characteristics or history, are at highest risk of development of neoplasia. Obviously, women who have never been screened should also receive priority for initial screening and outreach activities.

Comment

Despite aggressive screening and declines in cervical cancer mortality in the United States over the past four decades, between 1973 to 1974 and 1985 to 1986 mortality rates declined only 17% among women aged 50 years and older, compared with 43% for women under age 50, with rates in older black women persisting at nearly triple the rates in older white women.[54] Women aged 65 years and older continue to account for nearly one half these cervical cancer deaths.[55]

One of the most common reasons why women with invasive cervical cancer have not been detected at earlier stages, when treatment yields survival rates of 80% to 99%, is that they have never been screened.[56-61] This has been particularly true for elderly women.[57, 59, 61] The extension of Medicare coverage to cervical cancer screening will expand access to Papanicolaou testing to the growing elderly segment of our population and holds the potential, particularly for high-risk women such as the poor and racial minorities, to eliminate the avoidable morbidity and mortality from cervical cancer in this country.

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Toxicities in rats with free versus liposomal encapsulated cisplatin

Ronald K. Potkul, MD,[a] Javed Gondal, PhD,[b] Pincas Bitterman, MD,[c] Kenneth L. Dretchen, PhD,[d] and Aquilur Rahman, PhD[b]

Washington, D.C.

Wistar rats (five in each group) were given either 1 mg/kg of free cisplatin, 1 or 2 mg/kg of liposomal encapsulated cisplatin, or saline solution intraperitoneally biweekly for 15 injections. Rats in the free drug group showed significantly less weight gain; two rats died during the study. At necropsy, the free cisplatin–treated rats showed gross and microscopic evidence of peritoneal fibrosis that was not detected in any of the remaining groups. The free cisplatin–treated rats showed serum and histologic evidence of renal damage; all five rats had moderate or severe acute tubular necrosis. No renal abnormalities were detected in rats that received 1 mg/kg, and only focal or mild changes were found in rats that received 2 mg/kg of the liposomal preparation. Neurotoxicity, as determined by nerve conduction and inclined plane studies, developed in rats treated with free and liposomal cisplatin. These results are encouraging and warrant further investigation. (Am J Obstet Gynecol 1991;164:652-8.)

Key words: Cisplatin, liposomes, intraperitoneal chemotherapy

Ovarian cancer is the leading cause of death from gynecologic malignancies in the United States. It is estimated that there will be 20,500 new cases of ovarian cancer in 1990, which will eventually cause 12,400 deaths.[1] During the past 15 years, a major research effort has focused on ovarian cancer. Cisplatin (*SP*-4-2, diamminedichloroplatinum) has shown extensive activity against ovarian tumors, both alone and in combination with other agents. Initial response rates as high as 79% and 98% with cisplatin-containing regimens[2, 3] most patients are not cured of disease, and further improvements in therapy are necessary.

Several studies have suggested a clinically important dose-response relationship with cisplatin. Bruckner et al.[4] and Ozols et al.[5] gave high-dose cisplatin (120 and 200 mg/m^2, respectively) to patients who no longer had response to low-dose cisplatin therapy or who had a recurrence and responses were observed. Although high-dose cisplatin was particularly effective in patients who previously had responses, responses were also observed in patients who had stable disease or progressive disease while they received conventional doses of the chemotherapeutic agent. Unfortunately, the renal toxicity and neurotoxicity of intravenous high-dose cisplatin was substantial. Thirty-two percent of the patients in the study of Ozols et al.[5] had a rise in serum creatinine level (mean 2.1 to 5.6 mg/dl). In 37% of the patients severe paresthesia developed and two of those 19 became wheelchair dependent, which limited the clinical usefulness of these dose escalations.

An alternate approach that takes advantage of the apparent dose-response relationship to cisplatin is intraperitoneal chemotherapy. Studies with intraperitoneal cisplatin have demonstrated a pharmacologic advantage (peak peritoneal level to plasma level) of 21 to 72 mg/dl.[6, 7] The ability to provide locally high concentrations of cisplatin to tumor cells is dependent on good drug distribution in the peritoneal cavity. Unfortunately, a chemically induced peritoneal sclerosis by cisplatin has limited the number of intraperitoneal cycles that can be administered.[8]

There is a growing body of preclinical and clinical evidence suggesting that the liposomal encapsulation of chemotherapeutic agents can decrease toxicity while antitumor activity is retained.[9-12] We have recently encapsulated cisplatin in our laboratory, and a preliminary study of the short-term exposure to the drug in rats demonstrated significantly less nephrotoxicity with no loss in therapeutic efficacy in the L1210 leukemia and NIHOVCAR human cell lines (unpublished data). The nephrotoxicity (blood urea nitrogen and creatinine evaluations) of liposomal cisplatin in rats that received

From the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology,[a] the Division of Clinical Pharmacology, Department of Medicine,[b] the Department of Pathology,[c] and the Department of Pharmacology,[d] Georgetown University School of Medicine and Lombardi Cancer Center.
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Reprint requests: Ronald K. Potkul, MD, 3800 Reservoir Road, NW, Washington DC 20007.
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6 mg/kg intravenously was significantly less than that observed with cisplatin (66 versus 172 and 1.2 versus 2.2 mg/dl for blood urea nitrogen and creatinine, respectively). The current study was undertaken to evaluate the nephrotoxicity, neurotoxicity, and peritoneal toxicity of long-term exposure of free and liposomal encapsulated cisplatin in rats when it is administered intraperitoneally.

Material and methods

Animals. Female Wistar rats, 11 to 13 weeks old, weighing 175 to 200 gm, were purchased from Charles River Breeding Laboratories, Inc. (Boston). All animals were maintained according to accredited procedures at the Georgetown University animal facility with institutional Georgetown University Animal Care and Use Committee approval, and were fed Purina Rat Chow (Ralston-Purina Co., St. Louis) and water as desired.

Liposomal preparation. Cisplatin was provided by the Biological Testing Branch, Developmental Therapeutic Program, Division of Cancer Treatment, National Cancer Institute, Bethesda. Phosphatidylcholine, phosphatidylserine and cholesterol were purchased from Sigma Chemical Co., St. Louis. Phosphatidylcholine, phosphatidylserine, and cholesterol in a 3:3.1:3.7 molar ratio were dissolved in chloroform/methanol (5:1 vol/vol), after which the organic solvents were evaporated in a rotating flask under a vacuum. Of all the lipids used, these lipids in the ratios cited produced the highest encapsulation of cisplatin (35% to 45%). Cisplatin (3 mg/ml) in a hydration buffer solution (0.25% sodium chloride and 5% mannitol) was added to the film of dried lipids and dispersed by sonication (Mettler Instrument Corp., Hightstown, N.J.) for 10 minutes, purged with nitrogen, and left overnight in a ground neck flask for hydration. The hydrated suspension was sonicated for 20 minutes at 37° C at output control No. 7 in a Cuphorn sonicator (model 380, Heat Systems Ultrasonics, Inc., Farmingdale, N.Y.) to form liposomes. The liposome suspensions were dialyzed against saline solution for 3 hours (three changes, 1 hour each) at room temperature to remove the unencapsulated cisplatin. The remaining platinum encapsulated within liposomes was measured with atomic absorption spectrophotometry as described previously.[13] Stability studies demonstrate a shelf life for the preparation of at least 2 weeks at 4° C.

Drug treatment. Cisplatin was dissolved in saline solution to a final concentration of 0.04 mg/ml for a 1 mg/kg body weight dose, and liposomal cisplatin was suspended in saline solution to final concentrations of 0.04 and 0.08 mg/ml for 1 and 2 mg/kg doses, respectively (volume of injection 2.5 ml/100 gm, 2.5% body weight). Female rats (five in each group) were treated with 1 mg/kg cisplatin and both 1 and 2 mg/kg liposomal cisplatin intraperitoneally biweekly for 7½ weeks for a total of 15 injections. Drug treatment was withheld if the rat showed signs of severe toxicity. Five control rats received saline solution alone. The rats were put to death at week 10. To reduce renal toxicity a chlorouresis was induced in all groups by pretreatment with subcutaneous furosemide (12.5 mg/kg).[14]

Neurotoxicity. Noninvasive nerve conduction studies were performed at weeks 9 and 10 according to the technique described by DeKoning et al.[15] Briefly, the rats were anesthetized with ketamine and 2% xylazine (50 mg/kg). The tibial nerves were stimulated at the ankle by a 0.1 msec square-wave pulse delivered by a stimulator (Grass Instrument Co., Quincy, Mass.). The M and H responses were recorded from the small muscles on the plantar surface of the hind foot by a surface-recording electrode on a digital storage scope. The M response occurs in reaction to stimulation of the α-motor fibers, whereas the H response reflects stimulation of the afferent proprioceptic Ia fibers, which in the spinal cord monosynaptically excite α-motor neurons. The animals were also tested on an inclined plane by placing them on a board that could be elevated slowly.[16] The mean angle at which the rat failed to remain on the plane was determined by five independent trials.

Serum and histologic evaluation. At week 10 blood was obtained by cardiac puncture into 5 ml tubes, and serum was separated by centrifugation in a centrifuge (Beckman Instruments, Inc., Fullerton, Calif.) at 1800 *g* for 20 minutes. Standard serum chemistries were performed by the Georgetown Hospital Clinical Chemistry Laboratory with a Hitachi 737 automatic analyzer (Boehrman and Mannheim Diagnostics, Indianapolis). At necropsy, the liver, kidneys, intestine, and sciatic nerve were harvested, fixed in 10% buffered formaldehyde, and embedded in paraffin; sections were routinely stained with hematoxylin and eosin and evaluated microscopically in a blinded manner by one of us (P.B.). The nerves were stained with Luxol (DuPont Co., Wilmington, Del.) fast blue. Acute tubular necrosis was diagnosed if dilated tubules, lined by flattened epithelium showing atypical nuclei and mitotic figures and hyaline casts, were present. Acute tubular necrosis was graded as mild (focal) if <10% of the kidney sections showed the above morphologic changes, moderate if >10% of the kidney sections but <25% showed morphologic changes, and severe if >25% of the sections showed necrotic changes. The liver and intestine peritoneal surfaces were evaluated for significant pathologic changes that included inflammation and fibrosis.

Statistical analysis. Statistical analysis was performed with the two-tailed Student *t* test with significance defined as $p < 0.01$. Nonparametric analysis was done by χ^2.

Table I. Renal function tests in rats receiving long-term treatment with free or liposomal encapsulated cisplatin

Group	*Blood urea nitrogen*	*Creatinine*	*Potassium*	*Uric acid*	*Phosphorus*
Free cisplatin (1 mg/kg)	16.7 ± 1.5	0.6 ± 0.1	9.1 ± 0.7*	3.1 ± 0.7*	11.1 ± 1.2*
Liposomal cisplatin					
1 mg/kg	18.8 ± 1.5	0.7 ± 0.04	5.0 ± 0.5	1.1 ± 0.3	7.7 ± 0.9
2 mg/kg	16.2 ± 2.9	0.7 ± 0.2	5.6 ± 0.8	1.0 ± 0.4	8.6 ± 0.6
Control (0.9% sodium chloride)	14.4 ± 1.7	0.6 ± 0.06	6.1 ± 0.9	1.2 ± 0.3	9.0 ± 0.8

Values are mean ± SD.
*Significant difference from values for other groups.

Results

General toxicity and weight changes. Rats in the cisplatin-treated group showed significantly less weight gain during the course of the study. Their average weight gain was 15 gm, as compared with 83, 69, and 63 gm for the control, 1 mg/kg liposomal cisplatin, and 2 mg/kg liposomal cisplatin groups, respectively ($p < 0.01$). Two of the five free cisplatin-treated rats appeared very ill during the study; less body fat and ascites necessitated withholding treatment at weeks 6 and 7. These two rats died during the study at weeks 7 and 9. None of the rats in the control or liposomal cisplatin–treated groups appeared ill, developed ascites, or died during the study period.

Clinical chemistry. At the end of the experiment serum was obtained from the 18 surviving rats. Evidence for nephrotoxicity with significantly elevated ($p < 0.01$) serum potassium, uric acid, and phosphorus levels was found in the rats given free cisplatin (Table I). These chemistry results were 9.1 mEq/L, 3.1 mg/dl, and 11.1 mg/dl as compared with 6.1 mEq/L, 1.2 mg/dl, and 9.0 mg/dl in the saline solution–treated rats. These values in the liposomal cisplatin groups were not significantly different from those in the saline solution controls. The mean serum concentrations of glucose, blood urea nitrogen, creatinine, bilirubin, total protein, triglycerides, cholesterol, sodium, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, lactic dehydrogenase, calcium, chloride, and bicarbonate were not different among the four different treatment groups.

Gross findings. At necropsy, three of the five rats in the cisplatin-treated group showed extensive serosal fibrosis of the peritoneal organs that precluded their identification (Fig. 1). A fourth rat demonstrated early fibrosis with thickening of the bowel serosal surfaces. The fifth free cisplatin–treated rat had no gross evidence of peritoneal fibrosis. There was no gross evidence of peritoneal fibrosis in either the control or the liposomal cisplatin groups (Fig. 2).

Microscopic histopathologic evaluations. The bowel disclosed extensive serosal fibrous adhesions in the cisplatin-treated rats. The hepatic capsule showed severe fibrous thickening in four of five rats (Fig. 3) and mild to moderate fibrous thickening in the remaining rat. No fibrosis was seen in any of the liposomal cisplatin treatment groups.

Acute tubular necrosis, which was characterized by dilated tubules that were lined by flattened epithelium showing occasional atypical nuclei and mitotic figures and some hyaline casts, represented the cardinal morphologic findings in the kidney. These morphologic features was produced by the administration of free cisplatin. Data on the incidence and severity of renal lesions are summarized in Table II. Moderate to severe acute tubular necrosis was observed in the kidneys from all five animals that were given free cisplatin (Fig. 4). In contrast, no abnormalities were found in any of the kidneys of the five rats treated with the equivalent dose (1 mg/kg) of the liposomal preparation. Doubling the dose of liposomal cisplatin (2 mg/kg) caused only focal or mild acute tubular necrosis in four of five rats. The fifth rat had no detectable abnormalities. The difference between the acute tubular necrosis scores in the groups given free and liposomal cisplatin was significant ($p < 0.05$).

No evidence of axonal degeneration or myelin breakdown could be detected in any of the study groups.

Neurotoxicity. Data on the mean H response and inclined plane are summarized in Table III. Both 1 and 2 weeks after completion of the 7½-week-drug regimen, the rats underwent neurologic testing by nerve conduction experiments and the inclined plane. At week 9 both the 1 mg/kg free group (three surviving rats) and the 1 mg/kg liposomal cisplatin group had significant slowing of nerve conduction. This improved rapidly and equaled that of the control group at week 10. In spite of this apparent improvement, the inclined plane experiment continued to be significantly abnormal. Unfortunately, because of equipment failure, the 2 mg/kg liposomal cisplatin group could not be tested at week 9, but the H response continued to be signif-



Fig. 1. Severe serosal fibrosis in a rat that received 1 mg/kg free cisplatin.

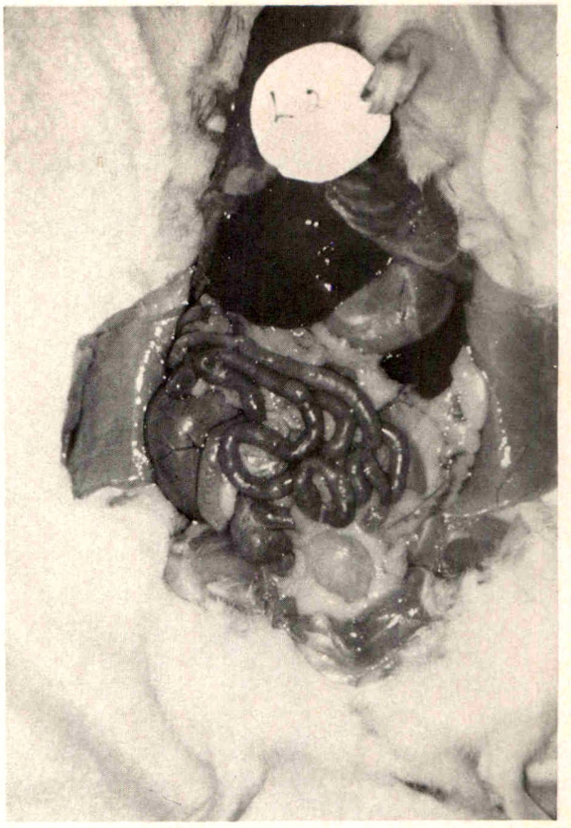


Fig. 2. No evidence of peritoneal fibrosis in a rat that received 2 mg/kg liposomal cisplatin.

icantly prolonged as compared with that of the other groups at week 10.

Comment

Drug carriers such as liposomes have been advocated as a potential means of ameliorating the toxicity of chemotherapeutic agents. This concept has been extensively studied in our laboratory by use of liposomal encapsulated doxorubicin and vincristine. In experiments that used a mouse model, liposomal doxorubicin was found to exert activity at doses that caused fewer myocardial alterations than did the same doses of free doxorubicin.[9] These findings were confirmed in a study of chronically administered doxorubicin in beagles and in a phase I clinical trial in which no cardiac toxicity was encountered, even at very high cumulative doses of the liposomal encapsulated drug.[10, 11] A similar decrease in toxicity is apparent in a liposomal preparation of vincristine.[12] In the mouse model, 3 mg/kg of free drug is lethal in 90% of the animals. A 7.5 mg/kg dose of liposomal vincristine was lethal in only 10% of the mice. Concurrently, we observed a significant improvement in the therapeutic efficacy of the liposomal preparation against L1210 leukemia as compared with the efficacy of the free drug at equal doses.

The difference in renal tissue damage induced by free and liposomal cisplatin was striking. In all of the animals receiving free cisplatin, moderate or severe acute tubular necrosis developed; this has been well documented in rats that received the free drug.[14] The effects of cisplatin in these animals are similar to the cardinal features induced in humans who receive cisplatin, namely, focal acute tubular necrosis, casts, dilated tubules, and epithelial atypia.[17] There was no evidence of acute tubular necrosis in rats that received the equal dose (1 mg/kg) of the liposomal preparation, and, in fact, only very minimal signs of acute tubular necrosis were found in the rats that received twice the dose (2 mg/kg). The serum chemistry results were somewhat confusing. Although the rats that received free cisplatin had significantly elevated uric acid, potassium, and phosphorus levels that were consistent with chronic renal insufficiency, blood urea nitrogen and creatinine levels were not significantly elevated. These rats appeared malnourished, with loss of body fat and muscle mass that could explain the normal cre-

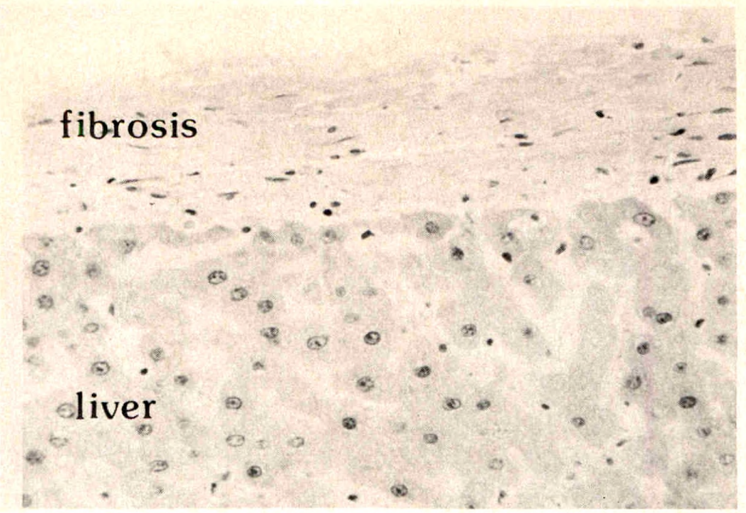


Fig. 3. Hepatic capsule showing severe fibrous thickening in a rat that received 1 mg/kg free cisplatin.

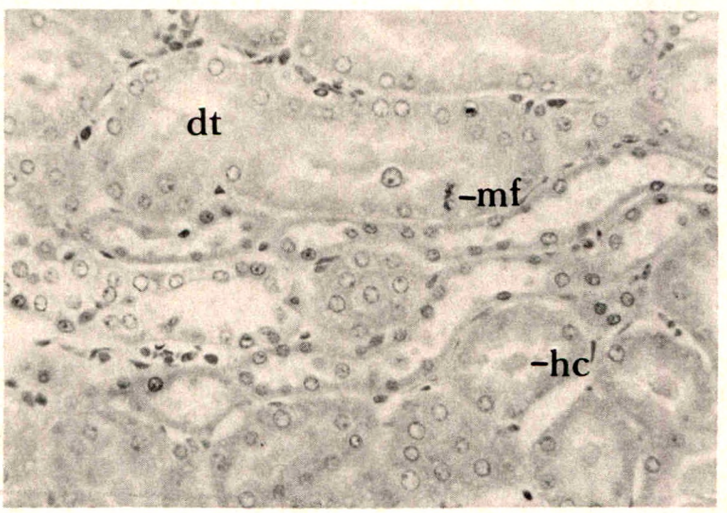


Fig. 4. Severe acute tubular necrosis characterized by dilated tubules *(dt)* lined by flattened epithelium showing occasional atypical nuclei, mitotic figures *(mf)*, and hyaline cases *(hc)* in a rat that received 1 mg/kg free cisplatin.

atinine despite acute tubular necrosis. The extensive peritoneal sclerosis may have limited food (i.e., protein) intake, thus lowering the serum blood urea nitrogen level in the animals with renal insufficiency.

A reduction in renal toxicity would be expected to be associated with other signs of protection, such as a decrease in the incidence of renal-related deaths. These factors were not directly addressed in the present study, but it should be noted that two of five rats that received free cisplatin (1 mg/kg) died, whereas all rats that received liposomal cisplatin (both 1 and 2 mg/kg) survived until the experiment was ended.

Liposomal encapsulation provided a means of altering other types of platinum toxicity. In this study peritoneal fibrosis developed in all rats that received free cisplatin intraperitoneally and obliterated the peritoneal cavity in three of five rats. Fibrosis was undetectable both grossly and histologically, even in the rats that received the high dose (2 mg/kg) of the liposomal preparation. We have previously shown in a phase I trial that doxorubicin can be safely administered intraperitoneally at significantly higher doses when it is encapsulated in liposomes.[18] Ozols et al.[19] encountered unacceptable chemical peritonitis with the administration of 15 to 20 μg/ml of free doxorubicin. We did not find this dose limiting toxicity with even 50 μg/ml of the

Table II. Comparison of the incidence and severity of acute tubular necrosis in rats receiving long-term treatment with either free or liposomal encapsulated cisplatin

Group	*Acute tubular necrosis*			
	None	*Mild*	*Moderate*	*Severe*
Free cisplatin (1 mg/kg)	0	0	3	2
Liposomal cisplatin				
1 mg/kg	5	0	0	0
2 mg/kg	1	4	0	0
Control (0.9% sodium chloride)	5	0	0	0

Table III. Comparison of the H response and inclined plane data in rats receiving long-term treatment with free or liposomal cisplatin

Group	*H response at wk 9 (msec)*	*H response at wk 10 (msec)*	*Inclined plane at wk 10 (degrees)*
Free cisplatin (1 mg/kg)	7.6 ± 0.3*	6.6 ± 0.2	65 ± 0.9*
Liposomal cisplatin			
1 mg/kg	7.4 ± 0.4*	6.3 ± 0.4	65 ± 1.0*
2 mg/kg	—	7.1 ± 0.4*	64 ± 2.6*
Control (0.9% sodium chloride)	7.0 ± 0.2	6.5 ± 0.3	72 ± 1.7

Values are mean ± SD.
*Significant difference from control value.

liposomal encapsulated drug. Peritoneal chemotherapy with its high peritoneal/serum ratio has many theoretical benefits. A large randomized phase III protocol (Southwest Oncology Group and Gynecologic Oncology Group) is attempting to answer the question of an actual clinical benefit of intraperitoneal versus intravenous cisplatin. If superiority of intraperitoneal administration is determined, this lack of fibrosis could have major clinical benefits by not limiting the number of intraperitoneal cycles that can be adequately delivered to the patient.

Damage to the sensory nerves that results in decreased conduction velocity and in sensory neuropathy has been reported in rats and humans who receive cisplatin.[15-20] The pathologic changes in peripheral nerves are most marked distally, which can explain the lack of abnormalities in the sciatic nerves of rats with severe neuropathy. Our comparative evaluation did not show a decrease in neurotoxicity with the liposomal encapsulated drug as compared with the free drug. However, using the same model system we have demonstrated a neuroprotective effect of the liposomal encapsulation of vincristine. The H responses were 5.5, 6.4, and 5.7 msec for the saline solution, free, and liposomal vincristine groups, respectively.[12] A larger experiment that more specifically examines dose-effect threshold and nerve recovery may show a difference between free and liposomal cisplatin.

In conclusion, the results of this study show that long-term intraperitoneal cisplatin-induced renal toxicity and peritoneal fibrosis can be markedly reduced by the administration of the liposomal preparation of this agent. Although these results are exciting, a detailed study is warranted to evaluate the role of liposomal cisplatin in the potential decrease of neurotoxicity associated with this important agent. The preliminary data indicate that this modality of treatment may have significant application in a clinical setting, and we believe that additional studies are warranted.

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Cocaine in pregnancy: The effect of maternal administration of cocaine on the maternal and fetal pituitary-adrenal axes

J.R. Owiny, PhD, M.T. Jones, PhD, D. Sadowsky, T. Myers, A. Massman, MD, and P.W. Nathanielsz, MD, PhD

Ithaca, New York

The effects of cocaine on the maternal and fetal pituitary-adrenal axis in vivo during pregnancy have not been reported. Six pregnant ewes and their fetuses underwent instrumentation at 113 to 119 days' gestation. Ewes were assigned to receive an intravenous bolus injection of vehicle or cocaine (0.5, 1.0, or 2.0 mg · kg^{-1}) at 124 to 136 days' gestation. Maternal arterial blood gases, fetal pH and fetal P_{CO_2} were unchanged after injection of cocaine or vehicle. After administration of 2.0 mg · kg^{-1} cocaine, arterial fetal P_{O_2} fell 3.2 ± 1.72 mm Hg ($p < 0.05$) at +5 minutes, returning to baseline by +15 minutes. Maternal and fetal adrenocorticotropin levels rose within 5 minutes after the highest cocaine dose ($p < 0.05$). There was a significant ($p < 0.05$) increase in maternal cortisol at all doses of cocaine and in fetal cortisol at +15 minutes after the 2.0 mg · kg^{-1}. (Am J Obstet Gynecol 1991;164:658-63.)

Key words: Cocaine, hypoxemia, ACTH, cortisol, sheep

Although cocaine abuse during pregnancy is an important clinical problem, few studies have been conducted to assess the mechanisms whereby short-term administration of cocaine can exert its harmful effects on mother and fetus. Clinically, these effects include increased incidence of premature birth, ruptured maternal intracranial aneurysms, intrauterine growth retardation, and abruptio placentae.[1, 2] Cocaine has been shown to cross the placental barrier in pregnancy.[3] Neonates exposed to cocaine in utero show abnormal behavioral responses, especially reductions in interactive behavior.[1]

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Reprint requests: Dr. Peter W. Nathanielsz, MD, PhD, Laboratory for Pregnancy and Newborn Research, NYS College of Veterinary Medicine, Dept. of Physiology, Cornell University, Ithaca, NY 14853.
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Cocaine blocks reuptake of catecholamines at nerve terminals, thereby increasing the level of catecholaminergic activity.[4] Catecholamines have been shown to decrease uterine circulation in pregnant sheep[5] and to lead to fetal hypoxemia.[6] Therefore cocaine may stimulate the fetal pituitary-adrenal axis secondarily to the induced fetal hypoxemia.[7] α-Adrenergic mechanisms may also stimulate arginine vasopressin release centrally,[8] which then acts to release adrenocorticotropin (ACTH) from the pituitary gland. Cocaine may also act more directly on maternal and fetal endocrine systems. In the rat, cocaine has been shown to stimulate ACTH release via a corticotropin-releasing factor–dependent mechanism.[9] These observations are in keeping with a stimulatory effect of cocaine on fetal plasma cortisol concentrations. However, in the fetal sheep inhibition of α-adrenergic receptors increases fetal plasma cortisol concentration threefold.[10]

In the sheep the fetal hypothalamic-pituitary-adrenal axis plays a fundamental role in the maturation of several organ systems, including the lung[11] and the hypothalamic-hypophysial-thyroid axis.[12] Cortisol that is secreted by the fetal adrenal as a result of stimulation by the fetal pituitary increases placental conversion of progesterone to estrogen, which eventually leads to labor and delivery.[13] In both human and nonhuman primates the maternal and fetal adrenals play a slightly different role in the process leading to parturition by providing C19 androgens to the placenta as precursors for estrogen synthesis.[14] In view of the importance of the maternal and fetal pituitary-adrenal axes in these critical processes in late pregnancy, we designed studies in the last third of pregnancy to identify the dose-related effects of cocaine on maternal and fetal secretion of ACTH and cortisol.

Material and methods

Care of animals and surgical procedures. Six Rambouillet-Columbia cross ewes, mated on a single occasion and carrying single fetuses of known gestational age, were used. All animals were weighed three to four days before surgery; mean weight was 68.9 ± 3.03 kg (mean ± SEM; range 60 to 78 kg). Surgery was performed at 113 to 119 days' gestation with animals under halothane anesthesia. Indwelling polyvinyl catheters were inserted into the fetal femoral artery and long pedal vein and into the maternal jugular vein and carotid artery.[7] All fetuses were allowed at least 5 days of recovery before undergoing any experimentation.

Administration of cocaine. Cocaine hydrochloride (Sigma Chemical Co., St. Louis) at 100% purity was used for all experiments. Doses of cocaine (0.5, 1.0, and 2.0 mg · kg^{-1} body weight) or vehicle (0.9% sodium chloride, wt/vol), were randomly assigned to the ewes. Each ewe received one injection of vehicle and one of each cocaine dose. Only one dose of cocaine or vehicle was given to each ewe on a single day. All experiments were conducted between 10 AM and 3 PM. Immediately before injection, cocaine was dissolved in 10 ml physiologic saline solution and administered into the maternal jugular vein over 30 seconds. Maternal and fetal arterial blood samples (3 ml) were taken at −30 and −20 minutes before the injection and at +5, +15, +30 and +60 minutes after the injection. The −30 and −20 values were averaged to provide a baseline value. Blood samples were drawn into cold disposable plastic syringes, immediately transferred to chilled heparinized 13 × 100 mm polypropylene tubes, and centrifuged at 4000 rpm for 5 minutes at 4° C. The plasma was transferred to plastic vials, immediately frozen in liquid nitrogen, and stored at −20° C until assayed. Maternal and fetal erythrocytes were aseptically returned after the last sample.

Analytic methods

ACTH. ACTH was measured by radioimmunoassay in unextracted plasma with a miniaturized version of the kit assay (INC Star, Stillwater, Minn.) previously described.[15] Briefly, 50 μl sample or standard was incubated with antihuman ACTH 1-24 antiserum (50 μl) for 24 hours at 4° C. Radioiodinated ACTH 1-24 (50 μl) was added and incubated for a further 24 hours at 4° C. Goat antirabbit precipitating complex (200 μl) was added and incubated for 20 minutes at room temperature (20° to 22° C). Interassay coefficients of variation for quality control samples containing 28.8 pg · ml^{-1} (fetal pool, $n = 9$) and 42.8 pg · ml^{-1} (maternal pool, $n = 8$) were 12.5% and 8.2%, respectively. Intrassay coefficients of variation for fetal and maternal pools were 6.1% and 3.0%, respectively. The limit of detection of this assay, that is, 90% B/BO, was 5.0 pg · ml^{-1}.

Cortisol. Plasma cortisol was measured (Autopak, Micromedics Systems Inc., Horsham, Pa.) as previously described.[15] Briefly, 20 μl samples were extracted with the methylene dichloride method and assayed in antibody-coated tubes with iodinated histamine-3-cortisol used as ligand. The intraassay coefficients of variation for this assay were 9.0% for maternal pool (mean concentration 68.7 ng · ml^{-1}, $n = 24$) and 10.0% for fetal pool (mean concentration 13.3 ng · ml^{-1}, $n = 10$). The interassay coefficient of variation for the maternal pool was 18.3% (mean concentration 45.5 ng · ml^{-1}, $n = 9$) and 22.6% for the fetal pool (mean concentration 19.5 ng · ml^{-1}, $n = 9$). The range of the standard curve was 0.96 to 64 ng · ml^{-1}. Assay sensitivity, calculated as 95% confidence of B/BO, was 0.96 ng · ml^{-1}.

Blood gases. Arterial blood samples were drawn anaerobically into 1.0 ml heparinized plastic syringes and analyzed for Po_2, Pco_2, and pH at 37° C with an ABL2 blood gas analyzer (Radiometer, Copenhagen).

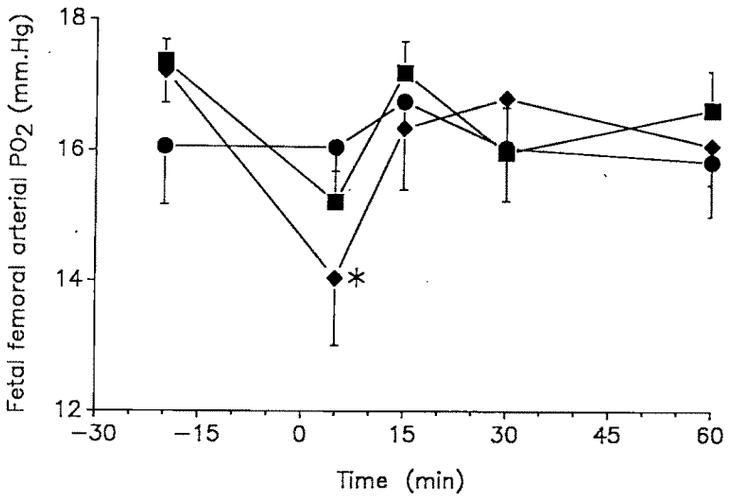


Fig. 1. Fetal femoral arterial P_{O_2} (mean ± SEM, $n = 6$) after injection of vehicle (●) or cocaine 1.0 mg · kg^{-1} (■) or 2.0 mg · kg^{-1} (◆) intravenously to pregnant ewes at 124 to 135 days' gestation. After the 2.0 mg · kg^{-1} dose fetal femoral arterial P_{O_2} at +5 minutes was less than the resting value (*asterisk*, $p < 0.05$).

Statistical analysis. To allow for individual animal variation all data were normalized at each study to the mean of the baseline values for that animal on the day of study. Analysis of variance was performed on each fetal and maternal variable to test for differences with time after injection of vehicle or each dose of cocaine. If initial analysis of variance showed a dose effect, changes within that dose were analyzed for time-related changes with the paired Student t test with Bonferroni correction performed on the normalized data. The level of significance was set at $p < 0.05$. The only significant change at the 0.5 mg · kg^{-1} dose was in the maternal plasma cortisol response. Therefore in the interests of clarity only data from the vehicle and the 1.0 and 2.0 mg · kg^{-1} injections are shown, although the 0.5 mg · kg^{-1} dose was used for the Bonferroni correction. Data throughout are presented as mean ± SEM.

Results

Outcome of experimental preparations. All fetuses were alive and well at the termination of the final cocaine administration. On the final day baseline fetal femoral arterial blood gases were P_{O_2}, 16.6 ± 0.46 mm Hg; P_{CO_2}, 45.5 ± 0.80 mm Hg; and pH, 7.4 ± 0.01. Maternal arterial blood gases were P_{O_2}, 99.3 ± 2.20 mm Hg; P_{CO_2}, 33.2 ± 1.14 mm Hg; and pH 7.5 ± 0.01.

Effect of cocaine on maternal and fetal blood gases

Maternal blood. Baseline maternal carotid arterial P_{O_2}, P_{CO_2}, and pH values did not differ during the study. Within each group maternal P_{O_2}, P_{CO_2}, and pH did not change over time after injection of vehicle or cocaine.

Fetal blood gases. Baseline values for fetal femoral arterial P_{O_2}, P_{CO_2}, and pH did not vary significantly on the 4 days of study. No changes were observed in fetal P_{CO_2} or pH after either vehicle or cocaine administration. There was a rapid transient reduction in fetal P_{O_2} after administration of cocaine, which returned to baseline by +15 minutes (Fig. 1).

Effect of cocaine on maternal plasma ACTH and cortisol concentrations. Fig. 2 shows changes in maternal plasma ACTH concentration after intravenous vehicle or cocaine administration to the ewe. Maternal plasma ACTH concentration at +5 minutes after the 2.0 mg · kg^{-1} dose was greater than baseline and +60-minute values. By +60 minutes maternal ACTH was back to baseline levels.

Maternal plasma cortisol concentrations after vehicle or cocaine administration to the ewe are shown in Fig. 2. At the 0.5 mg · kg^{-1} dose maternal cortisol was only significantly elevated at +15 minutes (12.0 ± 2.89 ng · ml^{-1} versus 7.9 ± 0.82 mg · ml^{-1} at baseline, $p < 0.05$). Maternal cortisol level was significantly elevated at +5 and +15 minutes after the two highest doses (Fig. 2).

Effect of cocaine on fetal plasma ACTH and cortisol concentrations. The fetal ACTH response to maternal injection of cocaine was variable (Fig. 3). At 2.0 mg · kg^{-1} the increase in fetal ACTH was significant only at +5 minutes. After vehicle alone there was an initial drop in fetal plasma cortisol at +5 minutes ($p < 0.05$). No other values differed significantly from baseline after vehicle injection. The only other significant change was an increase in fetal plasma cortisol at +15 minutes after the 2.0 mg · kg^{-1} dose (Fig. 3).

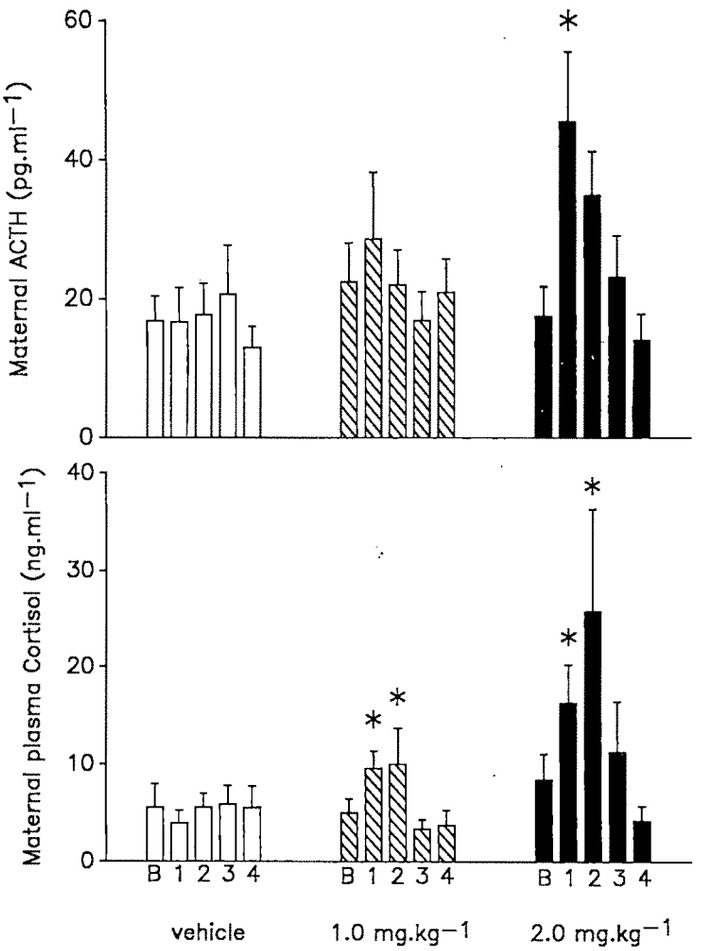


Fig. 2. Mean ± SEM ($n = 6$) maternal plasma ACTH and cortisol after injection of vehicle (□) or cocaine 1.0 mg · kg^{-1} (▨) or 2.0 mg · kg^{-1} (■) (*asterisk*, $p < 0.05$). *B* Represents the average of the samples at −30 and −20 minutes. *1*, *2*, *3*, and *4* Represent samples at +5, +15, +30, and +60 minutes, respectively.

Comment

We used the same doses of cocaine as Woods et al.[6] used in their study of the effect of cocaine on uterine blood flow in sheep in late pregnancy. At these doses the ewe showed some behavioral responses, including involuntary movements. The ewe often defecated at the lowest dose. On the basis of plasma concentrations of cocaine achieved in pregnant sheep after intravenous injection, the dose range of 0.3 to 1.0 mg · kg^{-1} corresponds to that customarily taken by addicted humans.[4] The maternal plasma levels obtained after injection of these doses were similar to the peak levels seen in human volunteers.[16]

As also reported by Woods et al.[6], we did not find any significant changes in maternal blood gas values after administration of vehicle or cocaine. However, there was a mild, transient fetal hypoxemia without alteration in fetal pH or P_{CO_2}. At the 2.0 mg · kg^{-1} dose the reduction in fetal P_{O_2} was significant at +5 minutes. The reduction in fetal oxygenation is most likely a result of decreased placental perfusion. Changes in fetal arterial P_{O_2} during decreased uterine blood flow are directly related to the degree of reduction in uteroplacental blood flow.[17] In the study of Woods et al.[6], a 2.0 mg · kg^{-1} dose of cocaine reduced uterine blood flow by 47%, because of a 168% increase in uterine vascular resistance with a fall in fetal P_{O_2} of about 5 mm Hg, returning to baseline in 15 minutes. We observed similar changes in P_{O_2}.

Administration of 2.0 mg · kg^{-1} cocaine led to a rapid increase in maternal plasma ACTH with a return to baseline by +60 minutes. The increase in maternal ACTH may be due to a direct action of cocaine on the maternal anterior pituitary[18] but is probably via a corticotropin releasing factor–dependent mechanism.[9] Maternal administration of cocaine in sheep is associ-

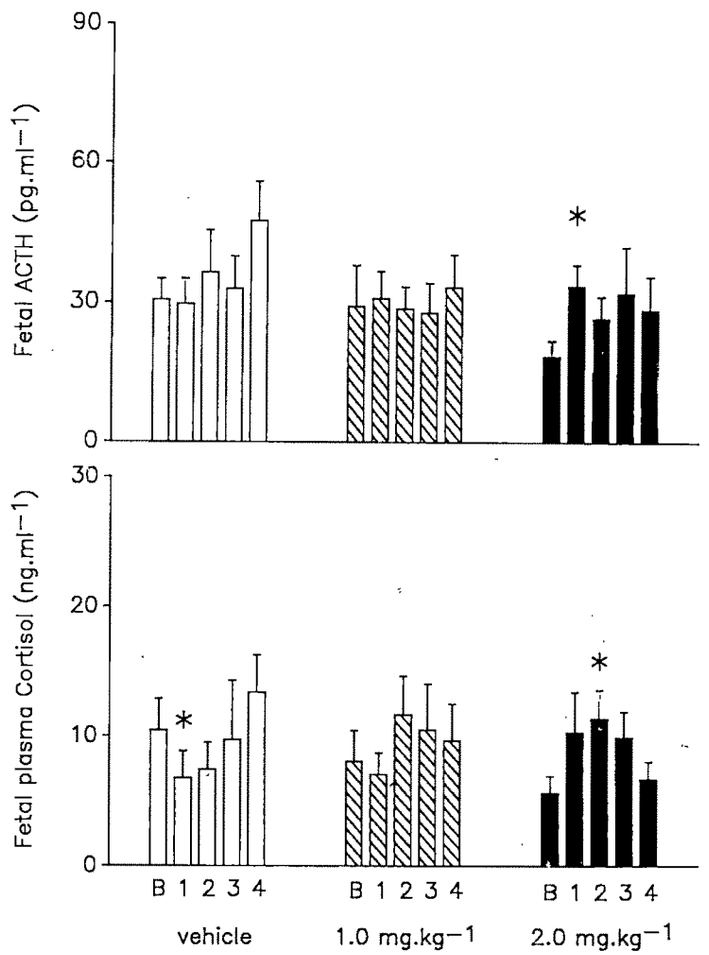


Fig. 3. Mean ± SEM ($n = 6$) fetal plasma ACTH and cortisol after injection of vehicle (□) or cocaine 1.0 mg · kg^{-1} (▨) or 2.0 mg · kg^{-1} (■) (*asterisk*, $p < 0.05$). *B* Represents average of samples at −30 and −20 minutes. *1, 2, 3,* and *4* Represent samples at +5, +15, +30, and +60 minutes, respectively.

ated with an increase in circulating catecholamines in both the ewe and the fetus.[4] The role of catecholamines in the regulation of ACTH secretion by sheep is unclear. Evidence exists for both stimulatory[19] and inhibitory[10] effects. In addition the release of catecholamines by cocaine may stimulate arginine vasopressin release.[8]

Maternal cortisol increased at all dose levels of cocaine. ACTH is normally released in a pulsatile fashion. The rise in maternal cortisol level is most likely explained by small undetected increases in maternal ACTH. The increase in maternal cortisol at the lowest dose of cocaine administered suggests that the maternal adrenal is probably sensitive to smaller rises in ACTH than can be detected significantly at our sampling frequency. Also cocaine may have a direct effect at the adrenal level on cortisol secretion.

The fetal ACTH response to maternal injection of cocaine was variable. In the 2.0 mg · kg^{-1} group there was a 95% increase in plasma ACTH concentration ($p < 0.05$) at +5 minutes. The large variability in the response may be related to the gestational age of the fetuses, as they were at a critical stage in the maturation of the hypothalamic-pituitary-adrenal axis. However, this increase in fetal plasma ACTH concentration is slightly larger than reported after comparable reductions in fetal oxygenation with oxytocin-induced uterine contracture.[20] The threshold for hypoxemia-induced fetal ACTH secretion is a 5 to 8 mm Hg reduction in carotid artery oxygen tension.[7] The level of fetal hypoxemia attained in our study (−3.2 ± 1.72 mm Hg) may not always have exceeded this threshold.

Cocaine may have a direct effect on fetal ACTH secretion. Although we did not measure plasma cocaine concentrations in either the mother or the fetus, it has been demonstrated that cocaine crosses the placenta rapidly after maternal injection[3, 4] and could possibly affect fetal ACTH secretion, especially at the highest

dose. Changes in fetal cortisol probably reflected the increase in fetal ACTH. The large variability seen in the response at the 0.5 mg · kg^{-1} dose suggests that this could have been around threshold for induction of cortisol release. It is also possible that an α-adrenergic discharge may have blunted some of the hypothalamic-pituitary-adrenal axis response to hypoxemia.[10]

In conclusion, we have demonstrated that intravenous administration of cocaine to ewes in late pregnancy is followed by an increase in maternal plasma ACTH and cortisol. The fetus responded with smaller increases in ACTH and cortisol. We speculate that these effects of cocaine during pregnancy represent changes in uterine blood flow leading to fetal hypoxemia together with direct stimulation of the hypothalamic-pituitary-adrenal axis. Further studies are needed to determine whether cocaine's effects are gestational age–dependent, especially later in pregnancy when the fetal adrenal gland is more sensitive to ACTH. In addition, information is required on the effects of long-term administration of cocaine on the maternal and fetal endocrine systems in pregnancy.

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Human amniotic fluid modulation of collagenase production in cultured fibroblasts

A model of fetal membrane rupture

Felipe Vadillo-Ortega, MD, PhD,[a] Georgina González-Avila, MD, MSc,[b] Carlos Villaneuva-Diaz, MD,[a] José Luis Bañales, BSc,[b] Moisés Selman-Lama, MD, MSc,[b] and Alberto Alvarado Durán, MD[a]

Mexico City, Mexico

The participation of a mechanical factor as the only cause of rupture of fetal membranes during normal labor or premature rupture has been criticized, and the involvement of an enzymatic mechanism has been proposed. In this study we analyzed the effect of human amniotic fluids at different gestational ages on the collagenase synthesis of cultured fibroblasts. Our results show that term amniotic fluids are capable of inducing the synthesis of collagenase and other proteases in fibroblasts, as revealed by selective increases in collagenase activity and in immunoreactive collagenase. Nonterm amniotic fluids failed to do the same. This phenomenon is proposed as a model for studying the collagen degradation of fetal membranes during term gestation. (Am J Obstet Gynecol 1991;164:664-8.)

Key words: Collagenase, amniotic fluid, fibroblasts, premature rupture of membranes

Fetal membranes rupture is an event usually associated with delivery. In normal circumstances these structures remain intact until advanced cervical dilatation,[1] but in some women the rupture is not associated with labor and even occurs in the absence of contractions. Under these conditions there may be mother-newborn complications because prematurity and infections are strongly related to premature rupture of membranes (PROM).[2, 3]

A mechanical factor such as the unique determinant of normal or abnormal rupture has been criticized[4, 5] and it has been suggested that an enzymatic mechanism may be involved. This resembles cervical ripening in which the active degradation of extracellular matrix components, mainly collagen, plays a well-documented role and is a prerequisite for cervical dilatation.[6-8]

Two recent articles[9, 10] reported the existence of local collagenolytic activity in fetal membranes. We showed in samples from PROM that there are extensive changes in collagen metabolism, including an increase in collagenolytic activity.[10] The existence of collagenolytic activity in fetal membranes allows us to postulate its possible participation in chorioamnion ripening. The activity of this system must be switched on around the moment of labor, and its action must produce a diminution of the tensile strength of amnion mediated by a decrease in the collagen content.

This article presents evidence that indicates there are factor(s) that appear in term normal amniotic fluid that modulates collagenase synthesis in cultured fibroblasts.

Material and methods

Amniotic fluid sampling. Seventy-two amniotic fluid samples were collected from 12 to 40 weeks' gestation. Gestational age was determined by the patient's history. The nonterm samples were obtained from pregnant women undergoing diagnostic amniocentesis for antenatal diagnosis of congenital abnormalities. All samples used were from women whose babies had no genetic alterations. Term gestation amniotic fluid samples (38 to 40 weeks) were obtained at the moment of delivery. Grossly bloody samples were discarded.

Amniotic fluids were centrifuged at 10,000 *g* for 30 minutes in cold, sterilized by filtration through a 0.22 μm membrane (Millipore, Bedford, Mass.) and stored at −70° C until used. Prior to use, protein determination was performed with Bradford's method.[11] Unless otherwise indicated all reagents used were purchased from Sigma Chemical Co., St. Louis.

Fibroblast culture. Normal human fibroblasts (MRC-5 strain), obtained from the American Type Culture Collection, were seeded in 96 well plates and in 25 cm^2 tissue culture flasks (Flow, McLean, Va.). Cells were

From the Departamento de Bioquímica, Departamento de Fisiología de la Reproducción, and División de Clínicas Especiales, Instituto Nacional de Perinatología,[a] and Unidad de Investigación, Instituto Nacional de Enfermedades Respiratorias, Secretaría de Salud.[b]
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Reprint requests: Dr. Felipe Vadillo-Ortega, Departamento de Bioquímica, Instituto Nacional de Perinatología, Montes Urales 800, Col. Lomas Virreyes, Mexico, D.F. C.P. Mexico 11000.
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grown to confluency in Earle's modified Eagle medium (Biofluids, Rockville, Md.) with 10% fetal calf serum added, 100 U/ml penicillin, 100 μg/ml streptomycin, and 250 ng/ml amphotericin B in 5% carbon dioxide and 95% air and a humid atmosphere for 5 to 7 days.

Before the experiments for collagenolytic activity or collagenase measurement were conducted, fetal calf serum was eliminated by replacing the culture media three times in 12 hours with fresh medium without fetal calf serum. During the last change, amniotic fluid was added at 15% to 20%, adjusting the volume by adding 300 μg of protein per milliliter of medium. Incubation was continued 24 hours under the same conditions. Each amniotic fluid sample was evaluated in quadruplicate. Four wells without addition of amniotic fluid were used as controls in each plate. As a positive control for stimulation of collagenase synthesis by fibroblasts, phorbol myristic acetate was used at a final concentration of 10 ng/ml.[12] The incubation was stopped by adding Triton X-100 at a final concentration of 0.1% and was maintained at 4° C for 2 hours. All supernatants were removed and each well or flask was individually assayed for enzymatic activity or enzyme measurement.

Collagenolytic activity. Enzyme activity was measured as described by Terato et al.[13] Type I collagen was extracted from human placenta with the pepsin-digestion method of Miller.[14] Radiolabeling of collagen was performed with a slight modification of Lefevere's method[15] with 5.0 mCi of acetic anhydride labeled with tritium (New England Nuclear, Boston, Mass.) instead of 25 mCi.

Labeled type I collagen (25 μg) with a specific activity of 800,000 disintegration per minute per milligram were incubated in duplicate for 24 hours with 25 μl of supernatant in each well. A buffer of Tris hydrochloride 50 mmol/L, pH 7.4, was added to achieve a final concentration of 0.15 mol/L sodium chloride 10 mmol/L calcium chloride, and 0.02% sodium azide. Collagen degradation was stopped by adding ethylenediaminetetraacetic acid at a final concentration of 80 mmol/L; 50% dioxane was added cold and samples were centrifuged at 10,000 *g* for 10 minutes. Supernatants were counted in a liquid scintillation spectrophotometer (Beckman LS-100, 59% efficiency). Control tubes were identical preparations with EDTA added at time zero. Collagenolytic activity was limited to EDTA-inhibitable tritium-labeled collagen degradation, and it was expressed as micrograms of degraded collagen per well per 24 hours.

The presence of latent collagenolytic activity was analyzed in 25 μl of supernatant preincubated for 30 minutes at room temperature with 10 μg/ml of L-1-Tosylamide-2-phenylethyl chloromethyl ketone-treated trypsin, then soybean trypsin inhibitor was added in tenfold excess (moles per liter). The optimum trypsin concentration for activation was documented previously with a range from 0.01 to 100 μg/ml at eight different points, as suggested by Bauer.[16] Another 25 μl aliquot was incubated in the presence of 1.0 mmol/L aminophenylmercuric acetate, a well-known collagenase activator.[17] These activated supernatants were incubated for collagenolytic activity as mentioned above.

Collagenolytic activity in each liquid used was assayed with the above methods.

A collagenase inhibitor has been isolated and well-characterized from amniotic fluid samples.[18, 19] To control the individual amniotic fluid inhibitory capacity on collagenolytic activity of media from stimulated fibroblasts, 7.5μg of amniotic fluid protein was incubated with 25 μl of unstimulated fibroblast medium that was previously activated with trypsin-limited proteolysis, and assayed for collagenase activity as described. This amount of amniotic fluid protein was equivalent to that used in the conditioning experiments. Inhibition was calculated and used for correction of collagenolytic activity in amniotic fluid–stimulated fibroblasts media.

Nonspecific protease activity. Heat-denatured tritium-labeled collagen was used as substrate for nonspecific protease activity with the method of Sunada.[20] Supernatant from each well 25 μl was incubated with 25 μg of substrate (20,000 counts per minute) at 37° C for 24 hours. Activity was expressed as micrograms of substrate degraded per 24 hours per well. Each amniotic fluid was also incubated alone, as a control, under these conditions.

Collagenase measurement. Media of amniotic fluid-conditioned and Triton X-100–treated fibroblasts cultured in flasks were collected and concentrated 10 times by ultrafiltration. Immunoreactive collagenase protein was quantified with the indirect inhibition enzyme-linked immunosorbent assay described by Cooper et al.[21] The enzyme for development of the standard curve and the specific antiserum were provided by Dr. Eugene Bauer from Stanford University.

Statistics. Statistical differences were assessed with the Mann-Whitney U test.

Results

Fibroblasts under basal conditions produced only latent collagenase, which could be fully activated with 10μg trypsin/ml. Activatable collagenolytic activity was 2.38 ± 1.32 μg of degraded collagen per well per 24 hours (n = 14). This basal activity was substracted from the results of all experimental wells. The immunoreactive collagenase in this media was 89.03 ± 9.79 ng/ml (n = 6). Fibroblast release of collagenase was 348.64 ± 36.32 ng/ml (n = 4) in response to stimulation by phorbyl myristic acetate.

Nonstimulated fibroblasts also released enzymes that degraded tritium-labeled gelatin. The values for this

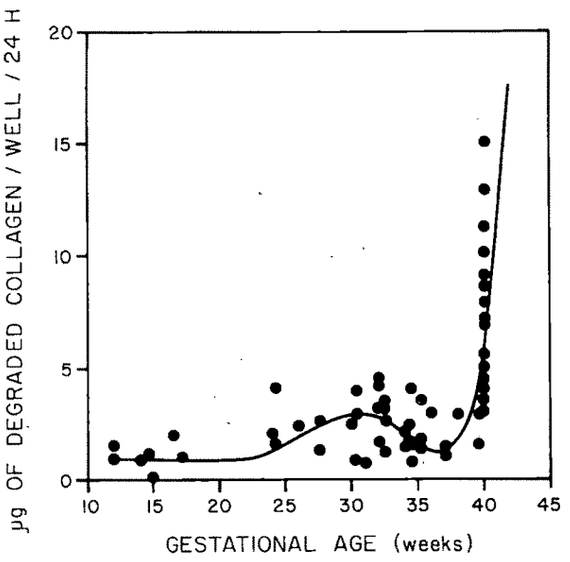


Fig. 1. Amniotic fluids from 12 to 40 weeks' gestation were added to fibroblasts in culture and incubated for 24 hours. Later, collagenolytic activity was assayed in the media. A clear stimulation of collagenase activity was observed when terminal amniotic fluids were used. All terminal amniotic fluids (38 to 40 weeks' gestation) are represented at point 40 on X axis.

enzymatic activity averaged 1.05 ± 2.32 µg of degraded gelatin per well per 24 hours (n = 14), and these values were substracted from degradation values in their respective experimental wells.

Amniotic fluids did not show collagenolytic activity at any gestational age. However, amniotic fluid from women ≥37 weeks' pregnancy had a nonspecific proteolytic activity against gelatin labeled with tritium. These values were used to correct the activity in their corresponding conditioned media.

We found some inhibitory effect of amniotic fluid on collagenolytic activity; however, it was not >5% under the experimental conditions. These individual values of inhibition were used to correct the collagenolytic activity in media of amniotic fluid-stimulated fibroblasts.

The addition of amniotic fluid to fibroblasts modified their basal production of collagenase and nonspecific proteases. A clear stimulation of these enzyme activities was observed when amniotic fluids captured during delivery from term gestations (38 to 40 weeks) were used (Figs. 1 and 2). In contrast, the use of amniotic fluid from nonterm, nondelivering gestations produced no effect because the values of active collagenase in media were similar to those from nonstimulated fibroblasts. Despite the fact that collagenolytic activity increased around week 30 (Fig. 1), the capacity for collagen degradation was very similar in all the media stimulated with nonterm amniotic fluid. Collagenolytic activity in media from fibroblasts stimulated with term amniotic fluid was 6.56 ± 3.15 µg of degraded collagen per well per 24 hours (n = 25). This activity was significantly higher ($p < 0.001$) when compared with the nonterm amniotic fluid induction (2.04 ± 1.11 µg degraded collagen, n = 47). Collagenolytic activity was in the active form in the media from amniotic fluid–stimulated fibroblasts and no further activation was obtained with trypsin or aminophenylmercuric acetate.

Measurements of immunoreactive collagenase showed a pattern that was very similar to the results from collagenolytic activity assay (Fig. 3). The media from nonterm, nondelivering amniotic fluid–induced fibroblasts contained 51.91 ± 25.86 ng collagenase per milliliter (n = 12), a value lower than the basal synthesis of nonstimulated fibroblasts ($p < 0.01$) and was clearly different from term amniotic fluid–stimulation (156.52 ± 32.37 ng/ml) ($p < 0.001$).

The pattern of nonspecific protease activity induction was similar to the collagenolytic activity. Term amniotic fluid–induced 11.35 ± 6.61 µg degraded tritium-labeled gelatin per well per 24 hours, whereas nonterm amniotic fluid only induced 0.67 ± 1.24 µg degraded tritium-labeled gelatin per well per 24 hours. These two groups were clearly different ($p < 0.0001$).

Comment

Degradation of extracellular matrix components requires a complex series of enzymatic activities. It is postulated that at the least three groups of proteases are involved in extracellular matrix catabolism. A group of enzymes degrades collagen-associated molecules such as proteoglycans and fibronectin and forms monomeric collagen. A second group directly attacks collagen and the third group hydrolyzes the collagen-degradation product until free aminoacids are obtained. Members of the first group are stromelysin and telopeptidase. The second group is formed by collagenases, a group of metalloenzymes with affinity for collagen that under physiologic conditions are a unique class of proteases capable of degrading native collagen. They are secreted in a latent form, and later, in the extracellular space, they are subjected to strict control. Its activity is dependent on complex interactions between the enzyme and inhibitors and activators. The last group of enzymes comprises several enzymes, such as gelatinase and PZ-peptide.[22, 23]

The results of this study reveal that amniotic fluids from term pregnancies contain chemical signals that enhance the in vitro synthesis of collagenase and other proteases in cultured fibroblasts. The fact that such effect had a clear association with the end of gestation suggests that it may play a role in vivo in the degradation of the extracellular matrix of fetal membranes during normal labor. Because preformed collagenase

is not stored inside fibroblasts,[24] the effect of amniotic fluid was possible because of an induction of collagenase synthesis, which is in agreement with the finding of increased enzymatic activity and immunoreactive protein quantity. On the other hand, the possibility of latent enzyme activation was controlled with the use of the collagenase activators aminophenylmercuric acetate and trypsin.

Amniotic fluids from nonterm gestations appear to inhibit the synthesis of collagenase as revealed by the immunoreactive measurement of this enzyme. However, its activity in media did not show this behavior, probably because nonlimiting conditions for enzyme activity were used.

The media from amniotic fluid-conditioned fibroblasts contained active collagenase only, as suggested by the fact that addition of the collagenase activators to the wells did not induce further collagenase activity. This could be explained by a collagenase activator already present in the system, which may be ascribed to the action of some of the induced nonspecific proteases. Characterization of these enzymes is mandatory to clarify this point.

The use of a fibroblast cell line as a model of the biochemical events that may occur in fetal membranes during labor is acceptable because this cellular population is a normal constituent of these tissues. We hypothesized that activation of local fibroblasts by a signal present in term amniotic fluid induces the synthesis of collagenase and other proteases. The subsequent degradation of collagen, which is the main structural component of membranes, diminishes the mechanical support of these tissues. At this moment the membranes are more fragile and a sudden increase in intrauterine pressure would lead to their rupture.

The identification of this mechanism opens the possibility of studying its participation in PROM because, as we have shown, in PROM there appears to be an increase in collagen degradation.[10]

It has been suggested that activation of local collagenolytic activity in amnion may be promoted by external factors such as inflammation and infection. In this context, it has been postulated that collagen degradation that occurs in PROM is caused by inflammatory cells, including polymorphonuclears and macrophages, through the synthesis of collagenase and other proteases.[4, 25] However, as suggested by this study, it is also possible that under normal or pathologic conditions, collagenolysis may be activated by local normally resident cells such as fibroblasts. It has been shown that fibroblasts respond to macrophage and lymphocyte signals with the synthesis of collagenase, stromelysin, and other connective tissue-degrading metalloproteases.[23, 26]

Characterization of the molecular nature of the compounds with activity on fibroblasts may be crucial in the search for the factors involved in normal, and probably abnormal, rupture of the membranes. A possible candidate for such a compound is prostaglandin E_2, be-

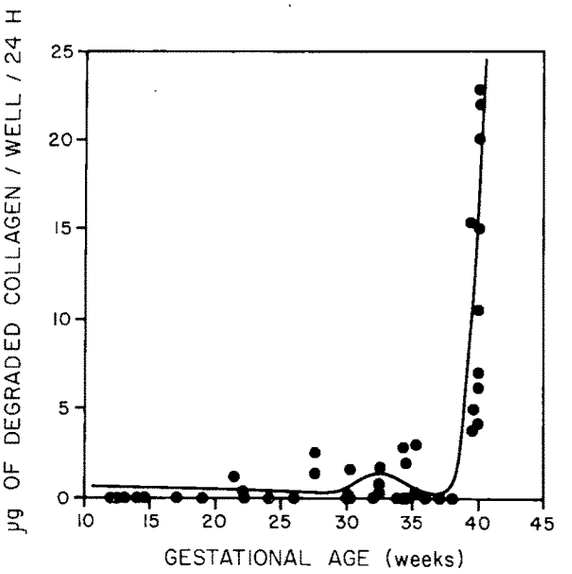


Fig. 2. Proteolytic activity against gelatin tagged with tritium was assayed in media of fibroblasts stimulated by amniotic fluid. Pattern of these proteases was similar to collagenolytic activity and showed peak of activity when terminal amniotic fluid was used. Terminal amniotic fluids (38 to 40) are grouped at point 40 on X axis.

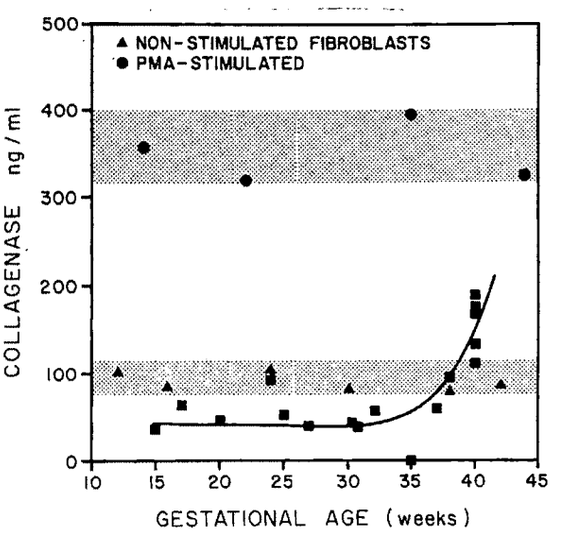


Fig. 3. Immunoreactive collagenase in media from fibroblasts stimulated by amniotic fluid was quantified with indirect inhibition enzyme-linked immunosorbent assay. Pattern of protein was similar to collagenolytic activity (■). As controls of fibroblast functionality, nonstimulated fibroblasts and phorbol myristic acetate–stimulated cells were included.

cause this molecule appears to be related to events in human labor under normal or abnormal circumstances, and it has been shown that this substance stimulates the production of collagenase.[27, 28]

The initiation of human labor is associated with mobilization of arachidonic acid from fetal membranes; and increased concentrations of prostaglandin E_2 in the amniotic fluid has been detected.[29] In addition, increased levels of this compound have been quantified in amniotic fluid of women with preterm labor,[30] and in the presence of infection, the latter mediated through endotoxins that stimulate amnion cells to synthesize prostaglandin E_2.[31] In this way it is possible to hypothesize that an unidentified signal in normal circumstances or bacterial products in pathologic conditions induce the synthesis of prostaglandins, especially prostaglandin E_2, and that this substance may then stimulate the synthesis of collagenase in local cell populations, which in turn allows PROM.

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Epidermal growth factor receptor expression in normal ovarian epithelium and ovarian cancer

I. Correlation of receptor expression with prognostic factors in patients with ovarian cancer

Andrew Berchuck, MD,[a, e] Gustavo C. Rodriguez, MD,[a, e] Ahmed Kamel, MD,[b, e] Richard K. Dodge, MS,[c, e] John T. Soper, MD,[a, e] Daniel L. Clarke-Pearson, MD,[a, e] and Robert C. Bast, Jr., MD[b, d, e]

Durham, North Carolina

Previous studies in breast and bladder cancer have suggested that epidermal growth factor receptor is expressed by only a proportion of cancers and is associated with poor clinical outcome. We used a monoclonal antibody specifically reactive with the extracellular domain of the epidermal growth factor receptor to localize this receptor immunohistochemically in frozen sections of normal ovary and epithelial ovarian cancer. Normal ovarian epithelium was found to express epidermal growth factor receptor in all cases. Among 87 ovarian cancers, however, 23% did not express immunohistochemically detectable receptor. Epidermal growth factor receptor expression was not related to histologic grade or stage, but was associated with poor survival ($p < 0.05$). The median length of survival of patients with tumors that did not express epidermal growth factor receptor was 40 months compared with 26 months in patients with tumors that did express epidermal growth factor receptor. As in breast and bladder cancer, expression of epidermal growth factor receptor in ovarian cancer appears to be a poor prognostic factor. (AM J OBSTET GYNECOL 1991;164:669-74.)

Key words: Epidermal growth factor receptor, epidermal growth factor, ovarian cancer

The epidermal growth factor receptor is a cell surface molecule that is widely distributed in normal human tissues. In vitro, after binding of one of its ligands, either epidermal growth factor or transforming growth factor-α, to the extracellular domain of the receptor, the tyrosine kinase of the intracytoplasmic domain is activated. This results in the generation of second messengers that transmit the mitogenic signal to the nucleus.[1] In vivo, the epidermal growth factor receptor and its ligands are thought to play a role in normal growth and differentiation although the precise nature of this involvement is not understood.

Abnormal expression of the epidermal growth factor receptor and its ligands has been associated with malignant transformation. Initially, it was noted that viral transformation of normal rat fibroblasts is associated with expression of transforming growth factor-α, which acts to stimulate growth by binding to epidermal growth factor receptors that are present on the same cells.[2] This phenomenon has been referred to as autocrine growth stimulation. Abnormal expression of epidermal growth factor receptor and its ligands also has been noted in human cancers. In breast cancer it has been shown that only one third of cases express detectable epidermal growth factor receptor, and that epidermal growth factor receptor expression is inversely related to estrogen receptor expression and associated with poor prognosis.[3, 4] Conversely, squamous cell cancers frequently overexpress epidermal growth factor receptor, although a direct relationship between overexpression and biologic behavior has not been demonstrated convincingly.[5, 6] Previously, we examined the relationship between epidermal growth factor receptor expression and prognostic factors in endometrial cancer.[7] We found that most, but not all, endometrial cancers express epidermal growth factor receptor, but no relationship to steroid receptor status or prognosis was noted.

Although epithelial ovarian cancer causes more deaths than all other gynecologic cancers combined,

From the Departments of Obstetrics and Gynecology, Division of Gynecologic Oncology,[a] Medicine,[b] Biostatistics,[c] and Microbiology-Immunology,[d] Duke University, and the Duke Comprehensive Cancer Center.[e]

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Reprint requests: Andrew Berchuck, MD, Duke University Medical Center, P.O. Box 3079, Durham, NC 27710.

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few studies have been performed to examine the relationship between epidermal growth factor receptor expression and the biologic behavior of ovarian cancer. In the present study, we used a monoclonal antibody reactive with the extracellular domain of the epidermal growth factor receptor to examine expression of this receptor in frozen sections of normal ovary and epithelial ovarian cancer. Epidermal growth factor receptor expression in the cancers was contrasted to that of normal epithelium, and the relationship between receptor expression and prognostic factors and survival in ovarian cancer was examined.

Material and methods

Patients. All the patients in this study with ovarian cancer underwent exploratory laparotomy in conjunction with treatment for epithelial ovarian cancer at Duke University between 1985 and 1989. Frozen tumor samples from 87 patients were found to be adequate for immunohistochemical analysis. Tumor was obtained at the initial operation in 57 cases (7 stage I or II, 50 stage III or IV). Among these 57 patients, five also had tumor samples frozen at second-look or subsequent laparotomy. In an additional 30 patients, tumor samples were obtained only at second-look laparotomy or at the time of recurrence (7 stage I or II, 23 stage III or IV). In addition, samples of normal ovary were frozen from six patients undergoing hysterectomy and bilateral salpingo-oophorectomy for benign gynecologic diseases.

All histologic material from the 57 patients in whom tumor samples were obtained at initial surgery was reviewed by a single pathologist (A.K.) and the histologic type (serous, mucinous, clear cell, endometrioid, undifferentiated) and histologic grade (well differentiated, moderately differentiated, poorly differentiated) were determined with the use of the World Health Organization criteria.[8] Most of the other 30 patients from whom tumor samples were obtained at a subsequent operation had undergone initial surgery elsewhere and slides were not available for review. However, in these 30 patients the diagnosis of epithelial ovarian cancer was confirmed by review of material obtained at a subsequent surgical procedure.

Immunohistochemistry. All tissue samples were snap-frozen and stored at −70° C until analyzed. Tissue samples were frozen in tissue Tek O.C.T. compound (Ames Division, Miles Laboratories, Elkhart, Ind.) and 4 to 6 μm thick cryosections were mounted on gelatin-coated slides. The slides were air dried overnight at room temperature to enhance the cellular morphology. The slides were fixed in acetone for 10 minutes at room temperature and then air dried again for 30 minutes. Then the slides were washed in phosphate-buffered saline solution three times for 5 minutes.

Immunohistochemical staining was performed with the Elite Vectastain ABC kit (Vector Laboratories, Burlingame, Calif.). Slides were placed in a humidified chamber and incubated for 15 minutes with several drops of a 1:200 dilution of horse serum in phosphate-buffered saline solution with 2% bovine serum albumin. Then, 70 μl of primary antibody was applied over the tissue sections for 60 minutes. After washing with phosphate-buffered saline solution, biotinylated goat antimouse immunoglobulin G (IgG) antibody was added for 30 minutes followed by the avidin-peroxidase complex. Finally, the slides were developed for 4 minutes with the enzyme substrate diaminobenzidine (0.5% diaminobenzidine in 0.05% Tris hydrochloride buffer, 0.6% hydrogen peroxide). The slides then were rinsed for 10 minutes in running tap water, counterstained with 1% methyl green (Sigma Chemical Co., St. Louis), dehydrated, and mounted.

Monoclonal antibodies. Purified 528 is a murine monoclonal antibody that is specifically reactive with the extracellular domain of the epidermal growth factor receptor. This antibody was generated by immunization of mice with A431 cells, which expressed markedly increased numbers of epidermal growth factor receptors[9] (528 was the generous gift of John Mendelsohn, Memorial Sloan-Kettering Cancer Center, New York). We tested 528 at concentrations of 0.01 to 5.0 μg/ml and determined that maximal immunohistochemical staining of the epidermal growth factor receptor was obtained with a concentration of 1.0 μg/ml. This concentration of antibody subsequently was used to stain all the tissue samples in this study. We used purified mouse IgG specific for nonhuman tissue (Coulter Immunology, Hialeah, Fla.) at a dilution of 1:100 as a negative control. Anticytokeratin AE1/AE3 antibodies (Boehringer-Mannheim Biochemicals, Indianapolis) were used as a positive control.

Immunohistochemical staining of the epidermal growth factor receptor was evaluated with serial sections. First, a section stained with hematoxylin and eosin was examined to evaluate the histology. Then a negative control slide stained with nonspecific mouse IgG was examined to assess nonspecific staining. A positive control slide stained with anticytokeratin antibodies was examined to confirm the presence of viable tumor cells in the section. Cases in which the negative or positive controls were not adequate were repeated. Finally, the slide in which 528 was used as the primary antibody was examined. The intensity of staining for epidermal growth factor receptor was graded as 0 (staining not greater than negative control), 1+ (light staining), 2+ (moderate staining), or 3+ (heavy staining). The intensity of staining recorded was the consensus of two observers who used a double-headed microscope. The identity of the patients was not known to the observers while the slides were being scored.

Statistics. Tables were analyzed with Fisher's two-

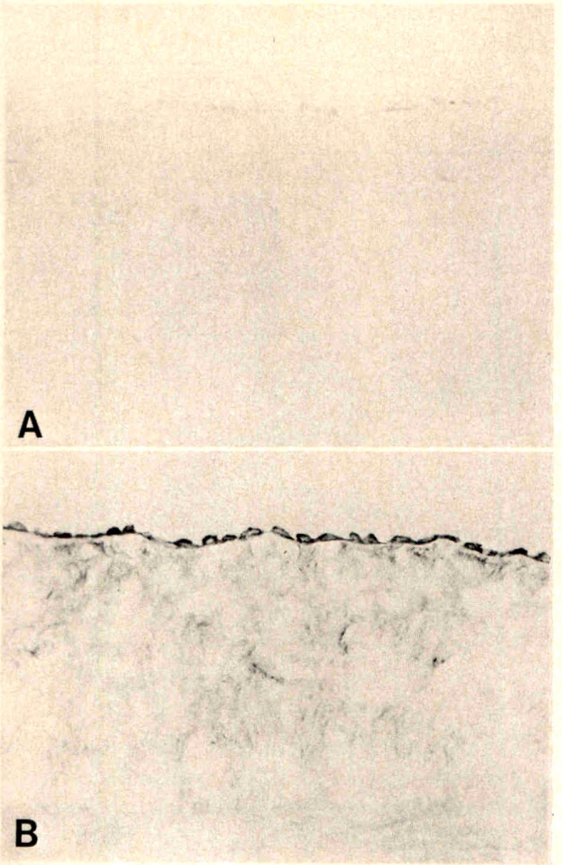


Fig. 1. Immunohistochemical staining of epidermal growth factor receptor in normal ovarian epithelium, **A,** negative control; **B,** epidermal growth factor receptor.

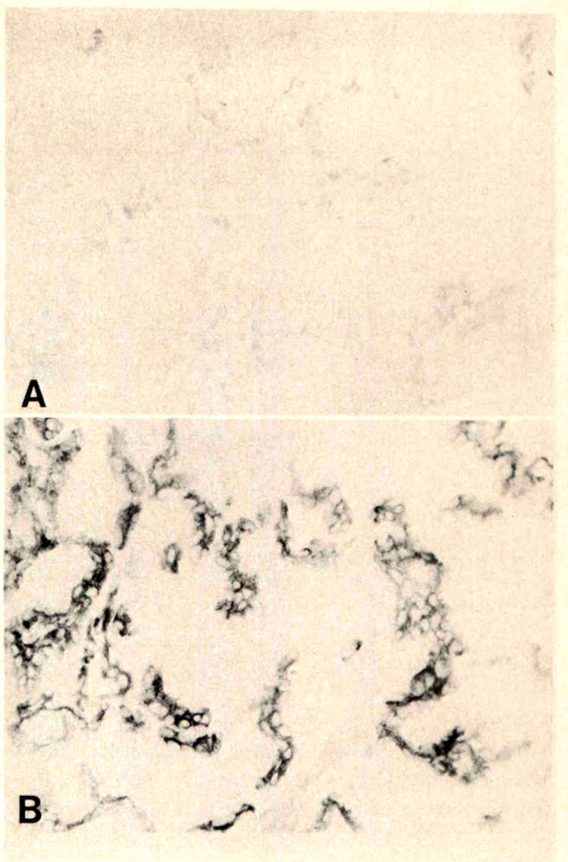


Fig. 2. Immunohistochemical staining of epidermal growth factor receptor in ovarian cancer, **A,** negative control; **B,** epidermal growth factor receptor.

tailed exact test. Survival estimates were calculated with the Kaplan-Meier life table method.[10] Differences in survival were tested with the log rank statistic.[11]

Results

Immunohistochemically detectable epidermal growth factor receptor was seen in normal ovarian epithelium in all cases examined. Homogeneous staining of all epithelial cells was noted in each case. In three cases, light staining was noted, and in three cases moderate staining was seen (Fig. 1, *A* and *B*). In all cases, lighter staining of the underlying stroma also was observed.

Among the 57 patients with ovarian cancer in whom tumor was frozen at initial surgery, 41 patients had serous tumors, 6 patients had mucinous tumors, 3 patients had endometrioid tumors, 2 patients had clear cell tumors, 4 patients had undifferentiated tumors, and 1 patient had a mixed serous-clear cell tumor. Among these 57 patients, cancer was well-differentiated in 6, moderately differentiated in 23, and poorly differentiated in 28. Staining for epidermal growth factor receptor was absent in 11 (19%), light in 18 (32%), moderate in 22 (38%), and heavy in 6 patients (11%). In addition to staining in the malignant epithelial tissue component, staining frequently was seen in the accompanying stroma (Fig. 2, *A* and *B*). In 11 patients with advanced-stage disease, tumor samples were obtained from multiple sites at the time of initial cytoreductive surgery. In these patients, epidermal growth factor receptor expression was evaluated in both the primary tumor and in one to three metastases. In all cases, the intensity of staining for epidermal growth factor receptor within a single patient was similar in the primary tumor and metastases. In addition, in five patients epidermal growth factor receptor expression was evaluated in tumor samples obtained both at initial surgery and at a subsequent laparotomy. In all cases, the intensity of staining for epidermal growth factor receptor was unchanged after therapy.

Because epidermal growth factor receptor expression did not appear to be altered during the course of treatment, we also analyzed epidermal growth factor receptor expression in an additional 30 tumor samples

Table I. Relationship between epidermal growth factor receptor expression and prognostic factors in advanced epithelial ovarian cancer

	EGF receptor–negative		*EGF receptor–positive*	
	No.	*%*	*No.*	*%*
Histologic grade				
Well differentiated	0	0	4	10
Moderately differentiated	3	33	17	41
Poorly differentiated	6	66	20	49
Cytoreduction				
Optimal (<1 cm)	3	23	6	16
Suboptimal (>1 cm)	10	77	31	84

EGF, Epidermal growth factor.

Table II. Relationship between epidermal growth factor receptor expression and findings at second-look laparotomy

			Second-look positive		
EGF receptor	*Unevaluable patients*	*Progression of disease*	*Gross*	*Microscopic*	*Second-look negative*
Absent	4	0	4	2	5
Present	13	9	23	5	8

EGF, Epidermal growth factor.

obtained from patients at the time of second-look or other subsequent laparotomy. Among these 30 patients, staining for epidermal growth factor receptor was absent in 9 (30%), light in 8 (27%), moderate in 13 (43%), and heavy in none (0%).

Overall, among all the patients in this study, epidermal growth factor receptor was absent in 20 of 87 cases of ovarian cancers studied (23%). Epidermal growth factor receptor was absent in 5 of 14 patients who had stage I or II disease (36%) compared with 15 of 73 patients who had stage III or IV disease (21%). In Table I, the relationship between epidermal growth factor receptor expression and other prognostic factors is presented for the 50 patients with stage III or IV disease in whom tumor samples were obtained at primary surgery. There was no significant relationship between epidermal growth factor receptor expression and histologic grade or the ability to perform an optimal cytoreductive operation that left no tumor nodule >1 cm in diameter.

Table II examines the relationship between epidermal growth factor receptor expression and response to primary therapy in the 73 patients with advanced-stage disease. Among the 15 patients whose tumors did not express epidermal growth factor receptor, 4 patients (27%) did not undergo second-look laparotomy because of patient refusal or poor medical condition. Among the 58 patients with tumors in which staining for epidermal growth factor receptor was seen, 13 (22%) did not undergo second-look laparotomy. Although 5 of 15 patients whose tumors did not express epidermal growth factor receptor achieved a negative second-look laparotomy (33%) compared with only 8 of 49 evaluable patients whose tumors did express epidermal growth factor receptor (16%), this difference was not statistically significant ($p = 0.1$).

In Fig. 3, the relationship between epidermal growth factor receptor expression and survival is shown. Among all 87 patients, survival of patients whose tumors did not express epidermal growth factor receptor was significantly better than that of patients whose tumors did express epidermal growth factor receptor ($p < 0.05$). The median survival of patients whose tumors did not express epidermal growth factor receptor was 40 months compared with 26 months in patients whose tumors did express epidermal growth factor receptor. If only the 73 patients with stage III or IV disease are considered, the survival advantage for epidermal growth factor receptor–negative cases was at the limit of statistical significance ($p = 0.06$). The median survival of patients whose tumors did not express epidermal growth factor receptor was 37 months compared with 21 months in patients whose tumors did express epidermal growth factor receptor.

Comment

Previously, it was shown that epidermal growth factor receptor is expressed by a wide range of normal human cells of both epithelial and mesenchymal origin. For example, in the uterus, we and others have shown that epidermal growth factor receptor is expressed by endometrial glands, endometrial stromal cells, and myo-

metrium.[7, 12] Similarly, in this study both the surface epithelium and underlying stroma of the ovary were found to express epidermal growth factor receptor. The physiologic role of epidermal growth factor receptor in the ovary remains unknown.

Studies of epidermal growth factor receptor expression in human cancers generally have found that epidermal growth factor receptor levels are increased in some squamous cell cancers, whereas a proportion of adenocarcinomas appear to have lost epidermal growth factor receptor relative to normal glandular epithelium. In some but not all studies, alterations in epidermal growth factor receptor expression were found to correlate with clinical outcome. For example, a group from Newcastle, England, found that absence of epidermal growth factor receptor is associated with favorable clinical outcome in patients with breast[3, 4] and bladder cancer.[13] Conversely, in lung[14] and endometrial[7] adenocarcinomas, no such relationship was noted. In squamous cell cancers of the lung, one group reported that those with the highest levels of epidermal growth factor receptor (greater than fivefold increased) have poor survival rates.[15] However, other studies of squamous cell cancers arising in the head and neck[6] and other sites have not confirmed these findings.

Although ovarian cancer causes more deaths than all other gynecologic cancers combined, little data have been reported with regard to epidermal growth factor receptor expression in ovarian cancer. A single study reported that high epidermal growth factor receptor expression in ovarian tumors correlated with a favorable prognosis.[16] However, in this study all adnexal tumors including epithelial, germ cell, and stromal tumors were analyzed together. In the present study, we examined epidermal growth factor receptor expression in a group of patients with epithelial ovarian cancer with the use of an immunohistochemical technique. Immunohistochemically detectable epidermal growth factor receptor was not seen in 23% of cases. This incidence of epidermal growth factor receptor negativity is similar to that found in other immunohistochemical studies of epidermal growth factor receptor in lung[14] and endometrial cancers.[7] On the other hand, studies that assess epidermal growth factor receptor expression by measuring binding of radiolabeled epidermal growth factor to tissue homogenates usually report a higher incidence of epidermal growth factor receptor negativity, for example, one half to two thirds in breast cancer.[3, 4] Some of the differences in conclusions between different studies of the same type of cancer may relate to these methodologic differences. Whereas binding assays are quantitative, it is clear that benign stromal elements in homogenized tumor samples contribute to epidermal growth factor receptor content. On the other hand, although immunohistochemical techniques allow direct assessment of epidermal growth factor receptor expression by cancer cells, evaluation of the intensity of staining is subjective.

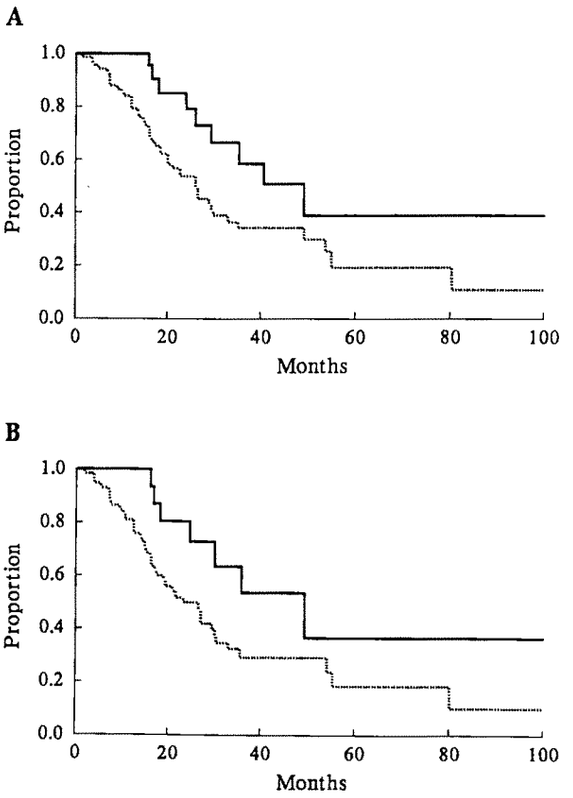


Fig. 3. Relationship between epidermal growth factor receptor expression and survival of patients with ovarian cancer (*solid line*, epidermal growth factor receptor negative; *dashed line*, epidermal growth factor receptor positive), **A**, all 87 patients ($p < 0.05$); **B**, 73 patients with advanced-stage disease ($p = 0.06$).

In this initial study, with the use of univariate analysis, we found a statistically significant relationship between epidermal growth factor receptor expression and survival in patients with epithelial ovarian cancer. Similar to breast and bladder cancer, absence of detectable epidermal growth factor receptor was associated with better long-term survival. Furthermore, although not statistically significant because of the small number of early stage patients, we found that absence of epidermal growth factor receptor was more common in stage I or II tumors (36%) compared with stage III or IV tumors (21%). This also is suggestive that absence of epidermal growth factor receptor is associated with cancers of lower biologic aggressiveness. Larger studies in which multivariate analysis can be used are needed to determine whether epidermal growth factor receptor status is an independent prognostic factor in ovarian cancer.

More recently, two other membrane-spanning

receptor-like molecules were discovered that have a high degree of homology to the epidermal growth factor receptor. The first, HER-2/neu (c-*erb* B2),[17] has been reported by Slamon[18] to be overexpressed in approximately 30% of breast and ovarian cancers. We confirmed the association between HER-2/neu overexpression and poor prognosis in ovarian cancer.[19] Both Slamon's study and ours found that the prognostic value of HER-2/neu overexpression in ovarian cancer was much stronger than that observed for epidermal growth factor receptor status in the present study. In addition, more recently, a third member of the *erb* B family (c-*erb* B3) has been identified.[20] It has been noted that c-*erb* B3, like c-*erb* B2, is overexpressed in some breast cancers. No studies of c-*erb* B3 expression in ovarian cancer have been performed to date. In the future, in addition to studies such as the present one that examine the prognostic significance of receptor expression, it will be critical to determine the actual biomolecular role of the c-*erb* B family of receptors in the process of malignant transformation of normal ovarian epithelium.

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Dose effects of antenatal corticosteroids for induction of lung maturation in preterm rabbits

Bannie L. Tabor, MD,[a] Evelyn D. Rider, MD,[b] Machiko Ikegami, MD, PhD,[b] Alan H. Jobe, MD, PhD,[b] and James F. Lewis, MD[b]

Torrance, California

Dose-response effects of corticosteroids were investigated in the preterm rabbit. Pregnant rabbits were given two doses of 0.01, 0.03, or 0.1 mg of betamethasone per kilogram every 24 hours, beginning on day 25 of gestation. Saline solution was used in a comparison treatment group. Half of the newborn rabbits received supplemental surfactant therapy after delivery via cesarean section on day 27, and all were ventilated on a ventilator-plethysmograph system for 30 minutes. The 0.01 and 0.03 mg/kg regimens had no effect on birth weight or lung function. The 0.1 mg/kg regimen resulted in fetal growth retardation and improved ventilatory measurements, gas exchange, and decreased protein accumulation in the lung, without increasing surfactant pool size. The induction of lung maturation by corticosteroids has multiple targets in the developing lung and does not exhibit a linear dose response. (AM J OBSTET GYNECOL 1991;164:675-81.)

Key words: Surfactant, pulmonary edema, compliance

Antenatal administration of corticosteroids has been used to induce pulmonary maturation and prevent respiratory distress in the preterm neonate.[1] Extensive laboratory and clinical studies have established the efficacy of corticosteroids, although the major effects of corticosteroids on the developing lung remain unclear. The preterm rabbit has served as a model for numerous investigations of lung maturation. Fetal growth retardation occurs in both rabbits and rodents with corticosteroid doses noted to alter lung development. The growth retardation is presumed to be due to effects on glycogen stores or decreased uteroplacental perfusion that is secondary to placental degeneration, resulting in decreased fetal oxygenation and substrate availability.[1] Most studies of lung maturation in preterm rabbits have used doses of betamethasone of 0.1 to 0.2 mg/kg maternal body weight, although a dose of 0.05 mg/kg was shown to have effects on lung maturation (increased dynamic compliance, improved pressure-volume relationships, and decreased protein leak into the lung) that were similar to those at higher doses.[2] The goal of this study was to identify the minimal effective corticosteroid dose that would influence lung maturation and to see if maturational responses could be dissociated from growth effects on the fetus.

From the Departments of Obstetrics and Gynecology[a] and Pediatrics,[b] Harbor–University of California, Los Angeles Medical Center.
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Reprint requests: Bannie Tabor, MD, Department of Obstetrics and Gynecology, Box 3, Harbor-UCLA Medical Center, 1000 W. Carson St., Torrance, CA 90509.
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Material and methods

Corticosteroid treatments. Time-bred New Zealand White rabbits were randomized to one of four treatment regimens beginning on day 25 of gestation (term = 31 days). The three hormonal treatment groups received maternal intramuscular injections of betamethasone (Celestone Soluspan; Schering Corp., Kenilworth, N.J.) at doses of 0.01, 0.03, and 0.1 mg/kg maternal body weight, respectively, every 24 hours for two doses. These doses were chosen because 0.1 mg/kg is known to be an effective dose, whereas 0.01 and 0.03 mg/kg were lower than the lowest dose of 0.05 mg/kg that has been shown to be effective.[2] A control group received 0.2 ml of saline solution on a similar dosing schedule. The guidelines for the care and use of animals approved by our institution were followed during the experiment.

Preparation of rabbits. On day 27 of gestation the pregnant does were given intravenous diazepam and supplemental oxygen via face mask and delivered via cesarean section with spinal anesthesia. The newborn rabbits were weighed and given 10 mg/kg ketamine and 0.1 mg/kg acepromazine by intraperitoneal injection. Alternate rabbits from each litter received 0.2 ml of 0.45% sodium chloride, containing either 1 μCi iodine 125–albumin or natural sheep surfactant (50 mg total lipid per kilogram) with 1 μCi ^{125}I-albumin. These suspensions were administered via a tracheal tube made from an 18-gauge needle, which was tied into the trachea.[3] Surfactant therapy was included in the protocol because synergistic action between surfactant treatment and antenatal corticosteroids has been noted in this model.

Table I. Summary of litters and fetuses studied

Group	Control	Betamethasone		
		0.01 mg/kg	0.03 mg/kg	0.1 mg/kg
Litters	5	4	4	8
Fetuses (No.)	47	30	34	65
Antenatal loss				
No.	1	0	0	21
Mean/litter (%)	2.0 ± 2.0	0	0	23 ± 10.3
Weight (gm)	28.2 ± 0.8	29.2 ± 0.9	28.0 ± 0.7	24.6 ± 0.7*
Trachea leak	3	2	0	4
Pneumothorax				
With surfactant treatment	0	0	0	0
Without surfactant treatment	7†	1	0	1
P_{CO_2} > 70 mm Hg (No.)				
With surfactant treatment	0	0	0	0
Without surfactant treatment	2	0	1	0
Animals used for analysis (No.)				
With surfactant treatment	18	13	16	19
Without surfactant treatment	16	14	17	20

*$p < 0.01$, versus all.
†$p < 0.05$, versus non-surfactant-treated groups.

Five breaths of 100% oxygen were given with an anesthesia bag to initiate ventilation after the intratracheal injection. The rabbits were then transferred to a ventilator-plethysmograph system that was temperature controlled at 37° C.[3] Initially, ventilation was given with 100% oxygen at a rate of 40 breaths per minute, an inspiratory time of 0.7 second, a peak inspiratory pressure of 35 cm H_2O, and an end-expiratory pressure of 0. Tidal volume was measured frequently with a pneumotachometer and maintained at 10 to 12 ml/kg by adjusting the individual peak inspiratory pressures.[3]

Each rabbit received 0.2 ml of saline solution containing 1 μCi iodine 131–albumin via the external jugular vein immediately after placement on the ventilator. The rabbits were ventilated for 30 minutes and then sacrificed with intrathecal injections of lidocaine. The chest was opened and a heparinized blood sample was drawn from the heart. An aliquot of this sample was used to measure P_{CO_2}. A second aliquot was used for measurements of labeled albumin and hemoglobin concentration. The sex of each rabbit was determined by examination of the internal gonads.[4]

Processing of samples. The lungs of the newborn rabbits were thoroughly lavaged with five aliquots (1 to 1.5 ml each) of 0.9% sodium chloride.[3] The pooled volume was recorded as the alveolar wash. The lungs were removed and weighed. The alveolar wash, lungs, and blood aliquots, along with triplicate samples of the ^{131}I and ^{125}I used for the initial injections, were counted for radioactivity. The lungs were then homogenized in 4 ml of 0.9% saline solution and the volume was recorded. The contribution of intravascular blood to the radioactivity associated with the lung was calculated by measuring the hemoglobin content and radioactivity of the blood and the lung and subtracting from the total radioactivity of the lung.[3]

Lipid extracts of aliquots of the alveolar wash and the lung homogenate were prepared with chloroform/methanol (2:1, vol/vol).[5] The extracts were treated with osmium tetroxide, and saturated phosphatidylcholine was isolated by alumina column chromatography and quantified by phosphorus assay.[6, 7]

Material. Radiolabeled albumin was prepared by iodination with carrier-free ^{131}I or ^{125}I and with the use of chloramine-T and monomer standard bovine serum albumin (Miles Laboratories, Elkhart, Ind.). The iodinated albumin was extensively dialyzed, and the incorporation was verified by trichloroacetic acid precipitation. The surfactant was isolated by a series of differential and sucrose density-gradient centrifugation steps from alveolar washes from adult sheep.[8]

Data analysis. Animals were excluded from the final analysis if upper airway leaks occurred during placement of the trachea tube, a pneumothorax occurred during ventilation, or the final P_{CO_2} was >70 mm Hg. The ^{125}I-albumin and ^{131}I-albumin radioactivity of the alveolar wash and the lung tissue was expressed as the percent recovery of the respective quantities injected. A final compliance value was calculated for each rabbit 30 minutes after the initiation of ventilation by dividing the measured tidal volume (in milliliters) by the peak inspiratory pressure (in centimeters of water) and the body weight (in kilograms). A ventilatory efficiency index was calculated for each rabbit as a measure of ventilation and gas exchange that compensates for the effects of ventilatory pressures, rate, and P_{CO_2}.[9] Ventilatory efficiency index = $3800/P \times F \times Paco_2$), where 3800 is a constant relating to carbon dioxide production, P is the peak inspiratory pressure minus positive

Table II. Ventilatory characteristics of newborn rabbits

Group	Control	Betamethasone		
		0.01 mg/kg	0.03 mg/kg	0.1 mg/kg
No surfactant treatment				
P_{CO_2} (mm Hg)	49.9 ± 3.5	47.8 ± 3.6	41.5 ± 2.0	42.9 ± 2.8
Mean tidal volume (ml/kg)	10.5 ± 0.2	10.1 ± 0.3	11.0 ± 0.3	10.5 ± 0.2
Mean peak inspiratory pressure (cm H_2O)	29.1 ± 0.6	30.9 ± 0.9	30.1 ± 0.7	26.4 ± 0.5*
Surfactant treatment				
P_{CO_2} (mm Hg)	39.4 ± 3.0	34.7 ± 3.0	40.6 ± 4.5	38.1 ± 2.9
Mean tidal volume (ml/kg)	10.5 ± 0.2	10.1 ± 0.2	10.7 ± 0.2	10.5 ± 0.1
Mean peak inspiratory pressure (cm H_2O)	18.2 ± 0.8	19.7 ± 1.4	21.6 ± 1.1†	15.9 ± 0.8‡

*$p < 0.01$, versus all non-surfactant-treated groups.
†$p < 0.05$, versus 0.01 mg/kg. $p < 0.01$ versus 0.03 mg/kg.
‡$p < 0.05$, versus control.

end-expiratory pressure, F is ventilatory frequency, and P_{aCO_2} is arterial carbon dioxide pressure. All values are reported as means ± SEM. Statistical analysis was performed with one-way analysis of variance followed by the Student–Newman-Keuls correction for multiple comparisons. Two-way analysis of variance was used to identify any sex-dependent interactions with treatment groups. No sex differences in any parameters were noted, so sexes within groups were combined for subsequent analysis. Significance was accepted at $p < 0.05$.

Results

Condition of animals. The number of litters and fetuses, mean birth weights of all live-born rabbits, and the number of animals excluded from analysis are described in Table I. The mean antenatal loss rate was higher in the litters receiving the 0.1 mg/kg betamethasone dosing regimen, but was not significant ($p > 0.1$). In addition, the mean birth weight in this group was significantly less than in the others ($p < 0.01$). The mean birth weights in the low-dose steroid groups were virtually identical to those of the control group. There were no differences in the incidence of tracheal leaks and final P_{CO_2} values >70 mm Hg. The only difference between the groups in terms of exclusion criteria occurred in the non-surfactant-treated groups, where the control group had a higher incidence of pneumothoraces ($p < 0.05$).

Ventilatory status. The ventilator strategy was successful in maintaining mean tidal volumes in the desired range for all treatment groups (Table II). The mean P_{CO_2} values were similar between the hormonal treatment regimens, when considered within the respective surfactant treatment groups. In the absence of supplemental surfactant therapy, the lower-dose steroid groups had mean peak inspiratory pressures similar to those of the control group, whereas the 0.1 mg/kg group required significantly lower pressures ($p < 0.01$). The control animals that sustained pneumothoraces had a mean peak inspiratory pressure of 32.0 ± 1.2 mm Hg at the time of the pneumothorax, which was similar to that of all but the 0.1 mg/kg group ($p < 0.01$). In the surfactant-treated groups the 0.1 mg/kg group had a lower mean peak inspiratory pressure than the 0.01 mg/kg ($p < 0.05$) and the 0.03 mg/kg ($p < 0.01$) groups but was not significantly lower than that of the control group. However, the 0.03 mg/kg group did require higher pressures than the control group ($p < 0.05$). Similar results were found in compliance (Fig. 1). Among the non-surfactant-treated groups the rabbits in the 0.1 mg/kg group were more compliant than the others ($p < 0.05$). With the addition of surfactant treatment, the highest-dose steroid group had higher compliance values than the others but the difference was significant only in comparison with the 0.01 and 0.03 mg/kg steroid groups ($p < 0.05$). The 0.1 mg/kg group also had a higher ventilatory efficiency index than the other groups, both with and without surfactant treatment, but this was significant only among the non-surfactant-treated rabbits ($p < 0.05$) (Fig. 1).

Surfactant pool size. The amount of saturated phosphatidylcholine in the alveolar wash and the total lung (lung homogenate plus alveolar wash) was measured to assess the effects on surfactant pool size. In the non-surfactant-treated groups there were no differences in terms of alveolar saturated phosphatidylcholine (Table III). However, treatment with the 0.1 mg/kg betamethasone regimen decreased the total lung surfactant pool ($p < 0.01$). In the surfactant-treated groups there were no differences in air space or total lung surfactant pool sizes.

Protein accumulation. The percent recovery of intravascular ^{131}I-albumin in the alveolar wash and total lung was used as a measurement of the accumulation of intravascular protein in the alveolus and lung tissue, with a higher percent recovery indicating greater protein leak into the lung (Fig. 2). In the animals not treated with surfactant, the 0.1 mg/kg dose of betamethasone decreased protein leak in both the airspaces

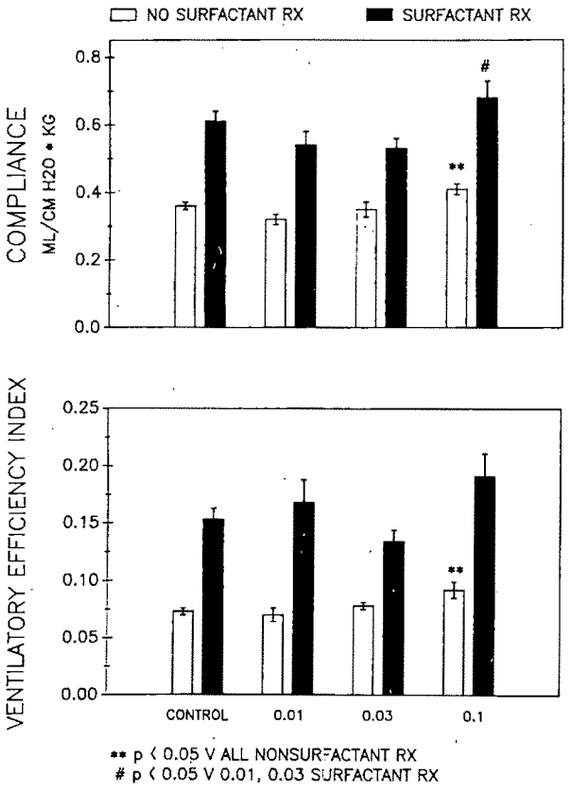


Fig. 1. Compliance *(upper)* and ventilatory efficiency index *(lower)* for corticosteroid treatment regimens with and without surfactant therapy. In this and subsequent figures 0.01, 0.03, and 0.1 refer to the doses of betamethasone in milligrams per kilogram. In the absence of surfactant therapy both compliance and ventilatory efficiency index were improved with the 0.1 mg/kg dose of betamethasone. With surfactant therapy the rabbits treated with 0.1 mg/kg were more compliant than those treated with 0.01 and 0.03 mg/kg.

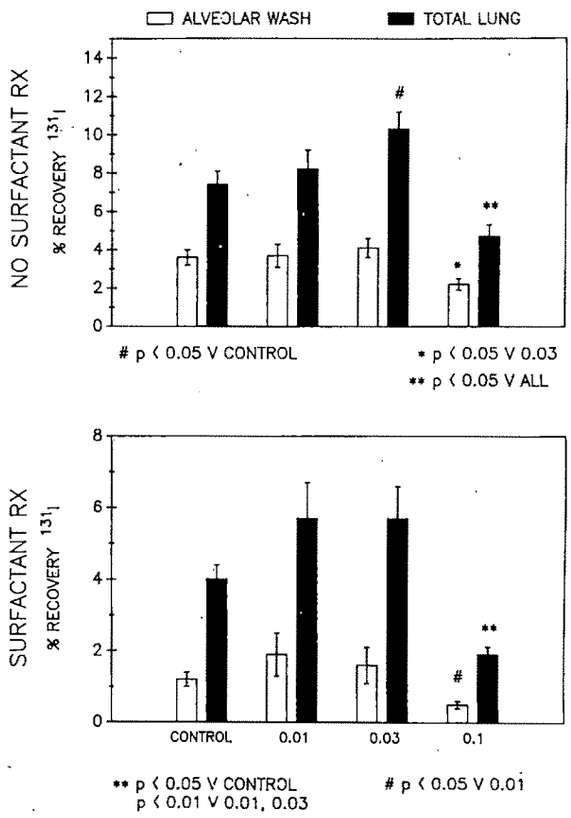


Fig. 2. Percent recovery of ^{131}I-albumin from the alveolar wash and total lung. In the absence of surfactant therapy *(upper)*, the 0.1 mg/kg dose had less total lung protein leak than the other doses. The 0.03 mg/kg dose had higher alveolar wash protein leak than the 0.1 mg/kg dose and higher total lung protein leak than the control animals. With surfactant therapy *(lower)* the 0.1 mg/kg dose had less total lung protein leak than the other doses and less alveolar wash leak than the 0.01 mg/kg dose.

Table III. Saturated phosphatidylcholine

Group	Control	*Betamethasone*		
		0.01 mg/kg	*0.03 mg/kg*	*0.1 mg/kg*
No surfactant treatment				
Alveolar wash (μmol/kg)	0.54 ± 0.05	0.49 ± 0.06	0.51 ± 0.07	0.45 ± 0.04
Total lung (μmol/kg)	59.0 ± 2.5	59.1 ± 2.9	60.0 ± 2.4	47.1 ± 2.5*
Surfactant treatment				
Alveolar wash (μmol/kg)	14.3 ± 2.2	13.2 ± 1.6	13.1 ± 2.2	13.7 ± 1.7
Total lung (μmol/kg)	80.6 ± 3.8	79.1 ± 3.6	78.8 ± 3.0	71.3 ± 3.4

*$p < 0.01$, versus all non-surfactant-treated groups.

(alveolar wash) and the total lung by 35% to 40% from the levels seen in the control animals, although this only reached significance with the total lung leak (total lung, $p < 0.05$; alveolar wash, $p < 0.1$). The protein leak in the 0.1 mg/kg group was also less than in the 0.03 and 0.01 mg/kg groups ($p < 0.05$). The alveolar wash leaks for the lower-dose steroid groups were similar to that of the control, but the total lung leak in the 0.03 mg/kg dose group was 40% higher than in the control ($p < 0.05$). Whereas the addition of surfactant treatment decreased the protein leak in all groups, similar relationships were found. The alveolar wash and total

lung leaks in the control group were twice those seen in the 0.1 mg/kg group, although again only the total lung leak was significant ($p < 0.05$). The protein leak into the air spaces and the total lung leak in both the lower-dose steroid groups were three times those of the 0.1 mg/kg group (total lung, $p < 0.01$ for both; alveolar wash, $p < 0.05$ for 0.01 mg/kg dose group).

The percent recovery of the intratracheal instilled ^{125}I-albumin reflects the movement of protein out of the airspaces, with a lower percent recovery indicating more movement, or leak, out of the lung (Fig. 3). In the non-surfactant-treated animals, the percent recovery was less in the 0.1 mg/kg group in the alveolar wash ($p < 0.01$ vs control) and in the total lung ($p < 0.05$ vs all). The addition of surfactant treatment increased the percent recovery in both the alveolar wash and the total lung and eliminated any differences between hormonal treatment groups.

Comment

This experiment illustrates the effects of antenatal corticosteroids in enhancing pulmonary maturation, as assessed in the preterm ventilated rabbit model. This model, with its varied end points, provides a more comprehensive picture of preterm lung function than can be obtained with in vitro studies, morphologic evaluations, or measurements of surfactant pool sizes and pressure-volume relationships in unventilated fetal lungs. In this model 0.1 mg betamethasone per kilogram maternal body weight improved overall lung function when given every 24 hours for 2 days before delivery. When comparison was made with the control group, this dosing regimen improved ventilation parameters (decreased peak inspiratory pressure and increased compliance) and gas exchange (increased ventilatory efficiency index), decreased protein leak into the lung, and improved protein clearance out of the airspaces and lung tissue. The lower doses of betamethasone did not have objective lung maturational effects. Most measurements were similar to those in the controls, the one exception being the incidence of pneumothorax in the non-surfactant-treated control animals. The lower doses of betamethasone may have induced subtle maturational changes in the lung tissue, which protected the airspaces from barotrauma at similar pressures. These changes were not evident in the more objective comparisons (protein leak, ventilatory efficiency index, or compliance) with the surviving control animals. In the surfactant-treated animals, which were ventilated at lower pressures, there were no differences between the low-dose steroid animals and the controls, including the incidence of pneumothorax. If anything, the lower doses tended to impair lung function, with greater degrees of protein leak and lower compliance.

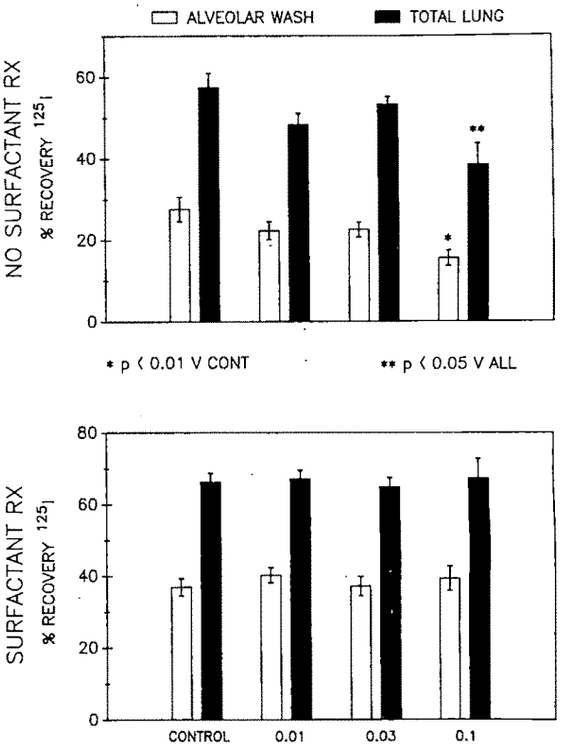


Fig. 3. Percent recovery of ^{125}I-albumin from alveolar wash and total lung. In absence of surfactant therapy *(upper)* improved protein clearance from alveolar wash (versus control) and total lung (versus all) was noted in 0.1 mg/kg group. No differences were noted in surfactant-treated groups *(lower)*.

Contrary to the perception that the beneficial effects of corticosteroids are due to stimulation of the surfactant system, these results were independent of surfactant effects. In the animals not treated with exogenous surfactant, the total lung saturated phosphatidylcholine pool was significantly less in the 0.1 mg/kg group. While studies in tissue cultures and unventilated fetal animals have shown increased surfactant synthesis with corticosteroids, our results are consistent with previous studies in the lamb and the ventilated preterm rabbit.[1, 2, 10] Since there is evidence suggesting the total lung saturated phosphatidylcholine pool may not accurately reflect the alveolar type II cell surfactant pool, the alveolar wash measurement may be a better marker of corticosteroid effects.[11] This tended to decrease with the highest dose of corticosteroid used. Corticosteroids may induce a more mature surfactant phospholipid and protein composition profile, which cannot be assessed by measuring only the saturated phosphatidylcholine pool.[12] We cannot comment on the degree of surfactant maturity in the high-dose steroid group, but the surfactant-independent effects of corticosteroids are further illustrated with exogenous surfactant treat-

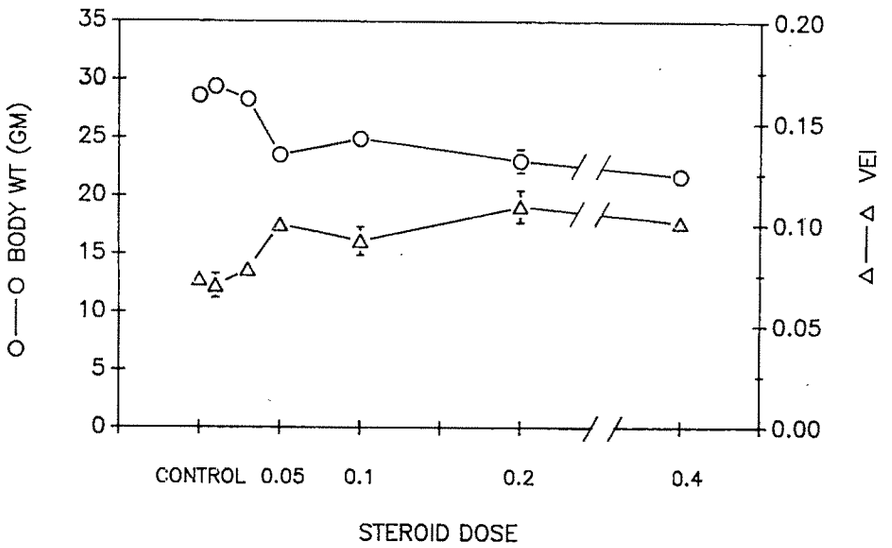


Fig. 4. Data from present study and that of Rider et al.[2] comparing body weight and ventilatory efficiency index for betamethasone doses ranging from 0.01 to 0.4 mg/kg. The 0.01 and 0.03 mg/kg doses had no effect on body weight or ventilatory efficiency index. The 0.05 mg/kg dose caused growth retardation and improvement in ventilatory efficiency index, with no changes in either parameter occurring with higher doses of betamethasone.

ment. The rabbits treated with 0.1 mg/kg betamethasone and surfactant were functionally more mature than the other surfactant-treated animals.

We could not separate the more objective pulmonary maturational effects of corticosteroids from the effects on growth retardation. Whereas lung maturation can be demonstrated in preterm mice and lambs without the detrimental impact on fetal growth, growth retardation and fetal loss are not uncommon in the preterm rabbit model.[2, 13, 14] The precise cause remains unclear. One possibility may be uteroplacental insufficiency as a result of placental degeneration. A second possibility is that the decreased growth is another direct effect of corticosteroids, via either depletion of glycogen stores or alterations in deoxyribonucleic acid synthesis and cell proliferation in sensitive preterm organs.[1]

When the results from this experiment are examined along with a previous investigation of corticosteroid treatments in rabbits, one sees a significant effect on fetal growth and lung maturation between doses of 0.03 and 0.05 mg/kg (Fig. 4).[2] Further enhancement of maturational effects, characterized in the figure by the ventilatory efficiency index, was not seen with doses exceeding 0.05 mg/kg. No growth effects were seen with 0.01 or 0.03 mg/kg, but similar degrees of growth retardation were seen beginning with the 0.05 mg/kg dose. These findings are consistent with the effects on fetal growth and pulmonary maturation being due to direct actions of corticosteroids and not secondarily due to placental degeneration. In the latter case one might expect varying degrees of uteroplacental insufficiency, growth retardation, and "stress-induced" lung maturation.

Corticosteroids act on target cells via specific receptors, which have been identified in fetal and adult lung tissue.[15] Whereas in vitro surfactant synthesis studies have demonstrated a linear relationship between glucocorticoid receptor occupancy and precursor incorporation, the few in vivo dose-response studies available are not so clear-cut.[16] Brehier et al.[17] found no effects on phosphatidic acid phosphatase activity in fetal rabbit lung with 0.025 mg betamethasone per kilogram of maternal body weight but similar induced enzyme activity with 0.1 and 0.25 mg/kg. Kaufman,[13] examining morphologic maturation in the preterm mouse lung, found maximal maturational changes with the lowest dose used (0.4 mg/kg) after 48 hours of therapy.

The observed dose effects add further credence to our suggestion that the beneficial effects derived from antenatal corticosteroids are not due primarily to the induction of surfactant synthesis. If this were the cause, by extrapolating from the in vitro studies, one would expect a somewhat linear dose response in overall lung function. Instead one sees an abrupt all-or-none response. The increase in compliance suggests alterations in the lung structure, perhaps changes in the collagen and elastin composition, as seen in the preterm lamb.[18] The function of the pulmonary vascular-alveolar barrier is improved, as evidenced by the decreased protein leak into and increased movement out of the airspaces,

as well as by the improved gas exchange suggested by the increase in ventilatory efficiency.

These findings offer some insight into the mechanism of action of corticosteroids in inducing preterm lung maturation. The impact of these agents on multiple sites that results in improved, more mature lung function should serve as a guideline for future investigation into the prevention and treatment of neonatal respiratory distress. It should also serve as a reminder that respiratory distress syndrome is not just surfactant deficiency.

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Phospholipase C activity in microorganisms associated with reproductive tract infection

James A. McGregor, MDCM,[a] David Lawellin, PhD,[b] Amalia Franco-Buff, MA,[b] and James K. Todd, MD,[b]

Denver, Colorado

Phospholipase C (lecithinase or phosphatidylcholine phosphorylase) catalyzes the hydrolysis of lecithin into phosphorylcholine and 1,2-diglyceride. Bacterial production of phospholipase C may damage reproductive tract tissues by both direct and indirect mechanisms. Use of the synthetic substrate p-nitrophenylphosphorylcholine polspholipase C activity was determined in 204 isolates representative of those found in female genital tract. Multiple aerobic (28%) and anaerobic (28%) reproductive tract microorganisms showed phospholipase C activity. Phospholipase C–producing isolates included strains of *Bacteroides fragilis*, *B. bivius*, *B. thetaiotaomicron*, *Gardnerella vaginalis*, and group B streptococcus. Phospholipase C activity was heterogenous; not all isolates that belong to a particular species showed activity. Phospholipase C production may be a possible virulence factor produced by a number of microflora commonly implicated in various reproductive tract infections or conditions, as well as in some instances of preterm birth. (Am J Obstet Gynecol 1991;164:682-6.)

Key words: Phospholipase C, bacteria, reproductive tract, virulence factor, preterm birth

Phospholipase C (lecithinase or phosphatidylcholine phosphorylase) catalyzes the hydrolysis of lecithin into phosphorylcholine and 1,2-diglyceride.[1] Bacterial phospholipase C was first described as α-toxin of *Clostridium perfringens*, and is well known as the lethal, hemolytic, and dermonecrotic factor responsible for the tissue destruction associated with this organism.[1] Phospholipids constitute the fluid matrix of most biologic membranes and phospholipid-hydrolyzing enzymes occur naturally in mammaliam and bacterial cells.[1, 2] Interest has been focused on phospholipase C as a possible bacterial virulence factor capable of mediating cell, tissue, organ, and whole organism toxic effects.[2-4]

Of special interest in reproductive medicine is the action of phospholipase C on lecithin to produce 1,2-diglyceride, which may further serve as substrate for production of arachidonic acid.[5] Arachidonic acid serves as substrate for the production of prostaglandins, thromboxanes, leukotrienes, and other eicosanoids. These biologically active substances play important roles in parturition, embryo implantation, coagulation, and inflammation. Phospholipase C may also directly disrupt cell integrity and cause release of biologically active cell contents into local tissues. Phospholipase C is thus a bacterial virulence factor that may cause both direct and indirect reproductive tract cell and tissue damage.

Numerous bacteria produce phospholipase Cs. Microorganisms in which phospholipase C production is extensively characterized include *C. perfringens*, *C. novyi*, *Staphylococcus aureus*, *Bacillus cereus*, and a number of strains of *Acinetobacter*, *Pseudomonas*, and *Proteus*.[1] Other organisms known to produce phospholipase C include a variety of other clostridial species, *Escherichia coli*, *Listeria monocytogenes*, *Ureaplasma urealyticum*, *Candida albicans*, *Serratia marcescens*, *Flavobacterium* species, a number of other *Enterobacteriaceae* and *Flavobacterium*, and several marine bacteria.[1-6] Production of phospholipase C has been suggested to be the crucial pathogenic mechanism among *Pseudomonas* strains that cause urinary tract infection[4] (i.e. phospholipases differ in details of their stereospecificity, optimum substrates, and growth conditions.[1, 2]). Until recently, such variability and the contamination of available substrates made examination for phospholipase C activity impractical. Kurioka's[7] development of the synthetic chromogenic substrate p-nitrophenyl-phosphorylcholine allows for reliable screening and measurement of phospholipase C activity produced by a variety of microorganisms grown in defined conditions.[4, 8]

Large collections of microorganisms of interest in human disease have not been systematically examined for phospholipase C activity. As part of a series of stud-

From the Departments of Obstetrics and Gynecology[a] and Microbiology-Immunology,[b] University of Colorado Health Sciences Center.

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Reprint requests: James A. McGregor, MDCM, University of Colorado Health Sciences Center, Box B198, 4200 E. 9th Ave., Denver, CO 80262.

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ies to determine the extent, sources, and possible importance of phospholipase C in reproductive tract infections in women, we evaluated phospholipase C activity in a collection of aerobic and anaerobic microorganisms recovered from patients with chorioamnionitis, postpartum infection, or acute salpingitis.

Material and methods

Cultures. Reproductive tract test microorganisms (128 aerobic and 76 anaerobic, Tables I and II) were obtained from 50 women at University Hospital, Denver, Colorado, who underwent microbiologic evaluation for postpartum endometritis, acute salpingitis, or chorioamnionitis. Aerobic and anaerobic cultures were obtained from endometrium with a sterile double-lumen collecting device after cleansing the cervix. Endocervical and fetal membrane (fetal side) cultures were obtained with sterile cotton swabs. Aerobic and anaerobic cultures were transported promptly to the microbiologic laboratory with Culturette collection tubes (American Scientific Products, McGaw Park, Ill.) or anaerobic specimen collection tubes (Vacutainer collection tubes, Becton Dickinson, Rutherford, N.J.), respectively.

Predominant aerobic (up to three) and anaerobic (up to two) isolates from each patient were selected for testing. Because of their importance in nonhuman reproductive tract infection, strains of *Brucella melitensis* and *Brucella suis* were obtained from previously collected and stored blood culture specimens. Otherwise, isolates were identified to a species level with standard techniques and maintained in glycerol at −70° C until reculturing on appropriate media and establishing purity before testing. Mycoplasma and chlamydia were not evaluated.

Cultures of aerobic organisms were grown overnight (18 hours) in tryptose minimal growth media while being agitated at 250 rpm on a reciprocating shaker (Gyrotory Shaker, model G2, New Brunswick Scientific, Edison, N.J.) at 37° C in 10% carbon dioxide. Anaerobic organisms were grown for 24 hours or until visible growth occured in tryptose minimal growth media within a Gas Pack (BBL Microbiology Systems, Cockneysville, Md.) at 37° C without agitation. Cultures were then adjusted to a final optical density of 0.1 at 590 nm before incubation with p-nitrophenylphosphorylcholine. Clinical findings and measurements of vaginal fluid phospholipase C activity were not correlated with microbiologic findings in this study.

Supplies. p-Nitrophenylphosphorylcholine was obtained from Sigma Chemical Company, St. Louis, Mo. Tryptose minimal growth media was used as media throughout and was prepared locally. Plastic microtiter plates were used as incubation vessels for 90 μl of tryptose minimal growth media mixed with 10 μl supernatant of test organisms, prepared to a concentration of 0.100 optical density at 590 nm. A Titertek microtiter plate reader (Flow Laboratories, Rockville, Md.) was used as described previously.[4]

Table I. Aerobe test specimens

	No. positive
Phospholipase C–producing	
B melitensis	1/1
B. suis	1/1
C. albicans	1/5
E. coli	*1/8*
G. vaginalis	2/7
M. morganii	3/3
N. gonorrhoeae	1/8
P. mirabilis	5/5*
P. vulgaris	2/2
P. aeruginosa	5/6*
S. saprophyticus	2/2*
S. epidermidis	4/15
Group B streptococcus	2/5
S. bovis	1/1
S. mutans	1/1
S. marcesans	1/1
α-Streptococci*	3/3
TOTAL	36/74(49%)
Non-phospholipase C–producing	
C. glabrata	0/9
Corynebacterium sp.	0/15
H. influenzae b	0/3
K. pneumoniae	0/1
L. monocytogenes	0/22
S. faecalis	0/2
S. mitis	0/1
S. pneumoniae	0/1
TOTAL	0/54

*Marked activity.

Enzyme assay. Ten μl of p-nitrophenylphosphorylcholine was added to each 100 μl of inoculated tryptose minimal growth media in sterile microtiter plates and read on a Titertek Mutiskan reader at 405 nm before incubation. The plates were sealed and incubated at 37° C for ten hours (aerobic organisms) or 24 hours (anaerobic organisms) without agitation. After incubation, the wells were reread with the microtiter plate reader. Controls included tryptose minimal growth media with or without 10 μl of p-nitrophenylphosphorylcholine. *Pseudomonas aeruginosa,* prototrophic strain PA01, whose metabolism in tryptose minimal growth media and phospholipase C action are established, was used as a positive control.[4-7] All tests were done in duplicate. Tests were interpreted as indicative of phospholipase C activity if both test postincubation colorimeter readings exceeded controls by 50%.[6]

Results

The 57 of 204 test specimens (28%) that showed phospholipase C activity are tabulated in Tables I and II. All PA01 prototroph stains of *P. aeruginosa* showed

Table II. Anaerobic test specimens

	No. positive
Phospholipase C–producing	
B. bivius	1/5
B. distasonis	1/5
B. fragilis	5/12
B. melaninogenicus	1/8
B. melaninogenicus-asaccharolyticus	1/2
B. thetaiotaomicron	4/5
F. mortiferum	2/2
Pc. sps.	2/5
Ps. anaerobius	1/1
*P. acnes**	3/5
TOTAL	21/50 (42%)
Non-phospholipase C–producing:	
Acetinomyces sp.	0/4
B. asaccharolyticus	0/2
B. melaninogenicus-asaccharolyticus	0/2
B. ovatus	0/2
B. ruminicola	0/1
B. uniformis	0/1
B. ureolyticus	0/1
B. vulgaris	0/4
E. corrodens	0/4
P. asaccharolyticus	0/2
P. magnus	0/1
V. parvula	0/2
TOTAL	0/26

*Marked activity.

phospholipase C activity. Previously documented phospholipase C activity was confirmed in our clinical isolates of *P. aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris*, *E. coli*, *S. marcescens*, *C. albicans*, and *Klebsiella pneumoniae*. On the other hand, our test isolates of *S. aureus* and *L. monocytogenes* did not show phospholipase C activity with p-nitrophenylphosphorylcholine substrate when grown in tryptose minimal growth media. Among anaerobic test isolates, microorganisms not previously examined or known for phospholipase C production include strains of *Staphylococcus epidermidis*, group B streptococcus, *Streptococcus mutans*, *Streptococcus faecalis*, and a nonhemolytic streptococcus. Anaerobic test organisms showing phospholipase C activity included a variety of *Bacteroides* species, including *Bacteroides thetaiotaomicron*, *Bacteroides fragilis*, *Bacteroides melaninogenicus*, *Bacteroides bivius*, *Bacteroides distasonis*, and *Bacteroides vulgatus*. Other organisms that showed phospholipase C activity included *Peptostreptococcus anaerobius* and *Fusobacterium mortiferum*. Anaerobic test organisms generally exhibited less phospholipase C activity than did aerobic bacteria under test conditions.

There was heterogenity of phospholipase C production within multiple isolates of the same species. Among species of test bacteria that showed phospholipase C production, 49% of aerobic and 42% of anaerobic test isolates showed activity. Because optimum culture and incubation circumstances for showing phospholipase C activity were not determined for each microbial species or test isolate, it is likely that a greater number of isolates may have shown phospholipase C activity if tested in more favorble test conditions or in various in vivo settings.[9] Several organisms commonly found in the genital tract could not be adequately grown in supplemented peptone broth and were not evaluated.

Comment

Phospholipase C activity occurs in a variety of anaerobic and aerobic microorganisms commonly isolated from various body sites in both health and disease. Addition of these newly noted phospholipase C–producing microorganisms to the list of bacteria previously recognized as producing phospholipase C suggests that a wide assortment of reproductive microorganisms is capable of phospholipase C production. Heterogeneity of phospholipase C production among genetically well-defined bacterial strains has been previously noted.[1-4]

Bacterial production of phospholipase C within the reproductive tract may damage the host in several ways. phospholipase C–mediated cell membrane hydrolysis may cause complete lysis of affected cells. Any cell may be disrupted, as is noted in the instance of *C. perfringens* septicemia. Bacterial phospholipase Cs may also act as hemolysins (*S. aureus* α-toxin). Phospholipase C may also mediate host damage by less direct mechanisms. Phospholipase C cell membrane hydrolysis, which does not necessarily cause cell lysis, releases phosphatidylcholine and 1,2-diglycerides. As noted, diglycerides can be converted into arachidonic acid by diglyceride lipase. Damaged, the remaining cell membrane is opened to other phospholipases that may further release arachidonic acid from exposed phospholipids. Lapetina et al.[10] suggest that phospholipase C may increase arachidonic acid production by converting phosphatidylinositol into phosphatidic acid, which is readily acted on by phospholipase A_1 to produce arachidonic acid.

Phospholipase C produced by various reproductive tract microorganisms may predispose patients to preterm birth by increasing local tissue concentrations of arachidonic acid and subsequent metabolites. Because prostaglandins and other eicosanoids are not stored within cells, availability of arachidonic acid is a primary rate limiting step in their production.[11] Pathophysiologically increased reproductive tract tissue levels may have numerous untoward effects. Prostaglandins E_2 and $F_{2\alpha}$ play primary roles in the initiation and maintenance of labor.[12] Both these substances may be used to pharmacologically initiate labor and abortion.[12]

Alteration of reproductive tract eicosanoid metabolism by phospholipase C–producing microorganisms may predispose or mediate labor less directly. Prostaglandin E_1 and thromboxane A_2 are chemotactic factors that incite and direct inflammatory cell migration.[11] Thromboxane A_2 is such a potent chemotactic sub-

stance that neutrophils may clog small vessels and impair microcirculation and tissue viability.[11] Neutrophils and macrophages in vessels and tisues may release damaging substances, such as collagenases, elastases, cathepsins, phospholipases, and free oxygen radicals that can further damage adjacent cells and extracellular connective tissue.[11] Release of such virulence factors within the cervix or lower uterine segment may be associated with direct collagenolysis and cervical softening or ripening.[13] Any of these substances may damage phospholipase A_2-laden decidual cells.[14] When disturbed, decidual cells can release phospholipase A_2 within the uterus, and act as a further stimulus to labor.[12]

Recent observations suggest that locally increased phospholipase C concentrations may further alter reproductive tract homeostasis at subcellular levels. Evidence now suggests that unsaturated diacylglycerols, released by endogenous or exogenous phospholipase C, act as physiologic agonists for activation of protein kinase C.[15-17] These effects may be blocked by phospholipase C inhibitors.[18] Friedman et al.[19, 20] first suggested that protein kinase C appears identical to epidermal growth factor receptor. This receptor is also activated by phorbol diesters.[18-20] Tumor-promoting phorbol diesters are, by themselves, noncarcinogenic but induce tumor formation when administered after subthreshold doses of a carcinogen.[21] Activation of protein kinase C by diglycerides, phorbol esters, and epidermal growth factor can induce multiple cell changes (i.e., morphologic transformation; altered prostaglandin, phospholipid, and protein synthesis; increased superoxide production; and loss of cell surface fibronectin and other cell surface proteins).[16-20] The bee phospholipase melittin promotes the same cellular effects as do phorbol esters.[21] It is of interest that recent work[22, 23] suggests that the oncogene *erb*B codes for a portion of epidermal growth factor receptor. The finding of this link between epidermal growth factor receptor gene and *erb*B underscores prior knowledge that receptors of other growth-stimulating agents, such as insulin and platelet-derived growth factor, are also kinases.[24] Growth modulating substances such as epidermal growth factor and other factors that activate cellular kinases are increasingly implicated in controlling the onset of human parturition.

These observations suggest that products of phospholipase C activity are basic to multicellular life and are consistent with activation of a protein kinase, most likely by enzyme phosphorylation in the presence of calcium.[15, 16, 18] Activation of cell protein kinase by diacylglyceride products of phospholipase C may occur in tissues normally exposed to phospholipase C–producing microorganisms, as well as those subject to phospholipase C activity during localized or systemic infection. Acute exposure to nonlethal concentrations of phospholipase C may alter both tissue and host defense cells in, as yet, undetermined ways. More prolonged exposure to bacterial phospholipase C produced by microorganisms may allow for an effect similar to those of phorbol esters in promoting transformation of cells also exposed to a carcinogen, thus possibly increasing risks of neoplasia within the lower genital tract.

Substantiation of in vivo bacterial phospholipase C production as a virulence factor within the reproductive tract can allow for the possibility of specifically mitigating phospholipase C activity. A variety of lower alcohols and univalent anions (Cl-, I-, and others) inhibit phospholipase C and other enzymes produced by *Bacillus cereus*.[22, 23] A synthetic compound, 2,3-distearoyioxyprolyl(dimethyl)-B-hydroxyethylammonium acetate, and the antimicrobials bleomycin and polymyxin B, inhibit release of phospholipase C, as do serine esterase inhibitors.[24, 25] Antibody to phospholipase C appears effective during in vitro neutralization tests, but no in vivo tests have been performed.[26] Addition of phosphate in vitro inhibits phospholipase C in a variety of *Pseudomonas* species.[7] More practically, subinhibitory levels of antimicrobials, such as clindamycin, inhibit production of extracellular bacterial products, including lipase production by *Propionibacterium acnes*. Treatment with even subtherapeutic amounts of selected antimicrobials that inhibit production or release of phospholipase C may be of benefit. Such observations allow speculation that microbial virulence factors such as phospholipase C may be inactivated or ameliorated by specific therapy if phospholipase C can be shown to be of significance in reproductive tract infection. Studies correlating vaginal concentrations of phospholipase C and pregnancy outcome are under way.

In summary, aerobic and anaerobic microorganisms frequently isolated from patients with reproductive tract infections were examined for phospholipase C production with p-nitrophenylphosphatidylcholine as substrate. Phospholipase C production was documented in vitro in numerous cervicovaginal aerobic and anaerobic microorganisms commonly involved in ascending reproductive tract infection (Tables I and II).

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The action of nitric oxide in the perfused human fetal-placental circulation

Leslie Myatt, PhD,[a, b, c] Anthony Brewer, BS,[a] and Diane E. Brockman, MS[a]
Cincinnati, Ohio

Nitric oxide is thought to be the endogenous endothelium-derived relaxing factor. We investigated the effects of compounds that either generate nitric oxide intracellularly or inhibit its action on the vasculature of the human placental villus. Addition to perfusion medium of methylene blue (10^{-5} mol/L), which is an inhibitor of activation of guanylate cyclase by nitric oxide, significantly increased perfusion pressure of the fetal-placental circulation over a range of flow rates (1 to 10 ml/min) compared with the perfusion pressures seen in the absence of methylene blue. This suggests basal release of nitric oxide may contribute to maintenance of resting vascular tone. Both glyceryl trinitrate (10^{-9} to 5×10^{-6} mol/L) and S-nitroso-N-acetylpenicillamine (10^{-8} to 10^{-4} mol/L), which generate nitric oxide intracellularly, were able to significantly vasodilate the fetal-placental circulation preconstricted with the thromboxane mimetic U46619 (1 to 5×10^{-8} mol/L) in a concentration-dependent manner. These compounds had no effect in the absence of the vasoconstrictor. Thus it appears that the placental villus vascular tree has the ability to both generate and respond to nitric oxide. (Am J Obstet Gynecol 1991;164:687-92.)

Key words: Nitric oxide, endothelium-derived relaxing factor, placenta, blood flow

Furchgott and Zawadski[1] first showed the necessity for the presence of endothelial cells in the relaxation of isolated rabbit aorta and other arteries induced by acetylcholine or other muscarinic agonists. A large number of agents are now known to give endothelium-dependent relaxation of blood vessels resulting from the release of a labile nonprostanoid endothelium-derived relaxing factor or factors.[2] Endothelium-derived relaxing factor acts by means of stimulation of guanylate cyclase in vascular smooth muscle to produce cyclic guanosine monophosphate, which causes relaxation. It is of interest that flow-induced shear stress also stimulates endothelium-derived relaxing factor release and endothelium-dependent relaxation occurs in resistance vessels as well as in arteries. During the same time period that endothelium-derived relaxing factor was first described, Ignarro et al.[3] found that nitric oxide caused a rapid transient relaxation of bovine coronary artery. Nitric oxide also activated soluble guanylate cyclase from coronary artery. The findings that both endothelium-derived relaxing factor and nitric oxide stimulate guanylate cyclase and that their action is inhibited by methylene blue, hemoglobin, and superoxide anion suggest a common identity. There is, however, evidence of other relaxing factors, including an endothelium-derived hyperpolarizing factor in some vessels.

In the absence of autonomic innervation, blood flow in the human fetal-placental circulation appears to be regulated by humoral agents or by some autocrine mechanism in the fetal-placental vasculature. We previously showed[4, 5, 6] that angiotensins I, II, and III and the thromboxane A_2 mimetic U46619 are vasoconstrictors in this circulation in vitro, whereas the vasodilator prostaglandins PGD_2, PGE_1, and $6\beta PGI_1$ (a prostacyclin analog) can attenuate angiotensin II–induced vasoconstriction. Van de Voorde et al.[7] showed that human umbilical blood vessel endothelial cells release an endothelium-derived relaxing factor in response to histamine but not acetylcholine, and Chaudhuri et al.[8] reported that both human umbilical artery and placental chorionic plate arteries were relaxed by substances that release nitric oxide. We decided therefore to establish whether the resistance vessels of the villous tree in human placental cotyledons were responsive to nitric oxide.

Material and methods

Placentas were collected immediately after normal vaginal delivery or elective cesarean section and transported to the perfusion laboratory in the labor and delivery area. A suitable third or fourth order chorionic artery and corresponding vein of an intact cotyledon were cannulated as previously described (Glance et al.,[4]

From the Departments of Obstetrics and Gynecology,[a] Physiology and Biophysics,[b] and Pediatrics,[c] University of Cincinnati, College of Medicine.
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Reprint requests: Leslie Myatt, PhD, Department of Obstetrics and Gynecology, University of Cincinnati, College of Medicine, 231 Bethesda Ave., Cincinnati, OH 45267.
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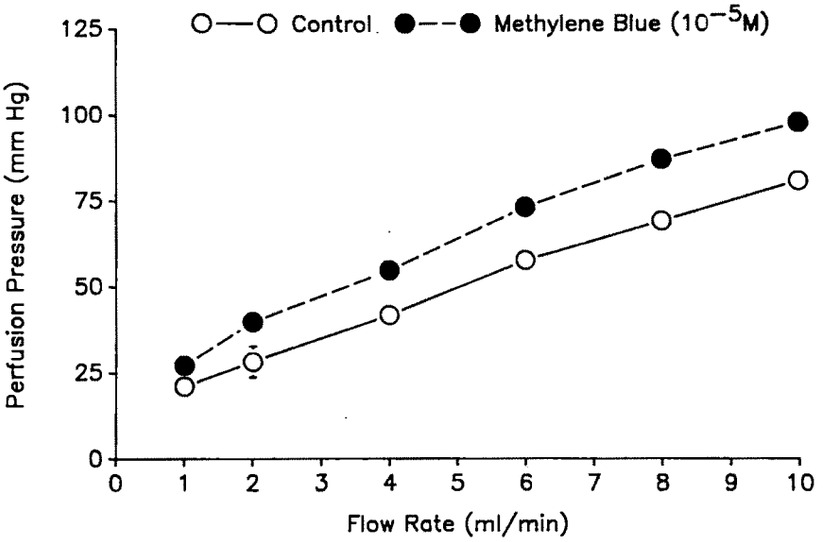


Fig. 1. Effect of methylene blue on perfusion pressure at differing flow rates. Perfusion pressure of fetal-placental circulation at varying flow rates (1 to 10 ml/min) was measured in same placenta in presence or absence of methylene blue (10^{-5} mol/L) in perfusion medium. The differences in perfusion pressure in presence or absence of methylene blue and at each flow rate were compared by two-way analysis of variance with repeated measures over two factors. Mean ± SEM of five separate experiments are shown (except where shown, SEM is smaller than size of symbol). There was significant difference between perfusion pressures obtained at each flow rate ($p < 0.005$) in either the presence or absence of methylene blue. Perfusion pressures in presence of methylene blue were significantly different from those in absence of methylene blue ($p < 0.005$) at each flow rate except 1 ml/min.

1984) at a point immediately before passage of the vessels through the chorionic plate. The maternal intervillous space was cannulated with two butterfly needles inserted through remnants of the spiral arteries in the basal plate. The effluent from the intervillous space was collected by gravity into an acrylic plastic cone over which the cotyledon was placed, maternal surface downward. Perfusion medium was tissue culture medium 199, containing 5% polyvinylpyrrolidone 40 and 0.1% bovine serum albumin as oncotic agents with 20 IU/ml heparin and 48 μg/ml gentamicin added, pH 7.4 with bicarbonate. Medium was gassed with 95% oxygen and 5% carbon dioxide at 37° C. Flow rates were 4 ml/min fetal and 10 ml/min maternal, except where stated. Lateral pressure was measured on fetal and maternal inflow lines adjacent to the point of cannulation. Data on pressure, inflow, and outflow Po_2 and pH were sampled every second and stored on an IBM PS2 computer system with Asystant Plus software (Asyst Technologies, Rochester, N.Y.).

In a series of experiments in which the involvement of a putative nitric oxide–mediated mechanism on flow-dependent vasodilation was investigated, the flow rate in the fetal circulation of the placenta was changed at 10-minute intervals in the order 4, 2, 4, 6, 8, 10, 4 ml/min. Perfusion pressure was measured throughout the experiment and maternal flow rate was kept constant at 10 ml/min. Then in the same placenta, methylene blue (10^{-5} mol/L) was added to both maternal and fetal perfusates and after a 20-minute period of equilibration, the flow rate was again changed at 10-minute intervals in the order 4, 2, 4, 6, 8, 10, 4 ml/min and perfusion pressure recorded. Both circulations were perfused with methylene blue throughout this entire sequence of flow-rate changes. In some experiments, perfusate was subsequently changed back to medium not containing methylene blue and, after an equilibration period of 20 minutes, the series of changes in flow rates was again performed and perfusion pressure measured at each flow rate.

The effects on fetal-placental perfusion pressure of agents that generate nitric oxide intracellularly was studied next. The thromboxane-mimetic U46619 was added to the fetal-placental perfusate in a final concentration range of 1 to 5 × 10^{-8} mol/L to increase fetal-placental perfusion pressure to 100 to 150 mm Hg at a flow rate of 4 ml/min. Then glyceryl trinitrate was injected as a 2 ml bolus in perfusion medium directly into the fetal inflow line, via an injection valve, over a range of concentrations (10^{-9} to 5 × 10^{-6} mol/L). Fifteen minutes were allowed to elapse between each infusion of glyceryl trinitrate to allow perfusion pressure to return to resting levels. The perfusates of both fetal and maternal circulations were then changed to fresh medium containing methylene blue (10^{-5} mol/L). A 20-minute period of equilibration

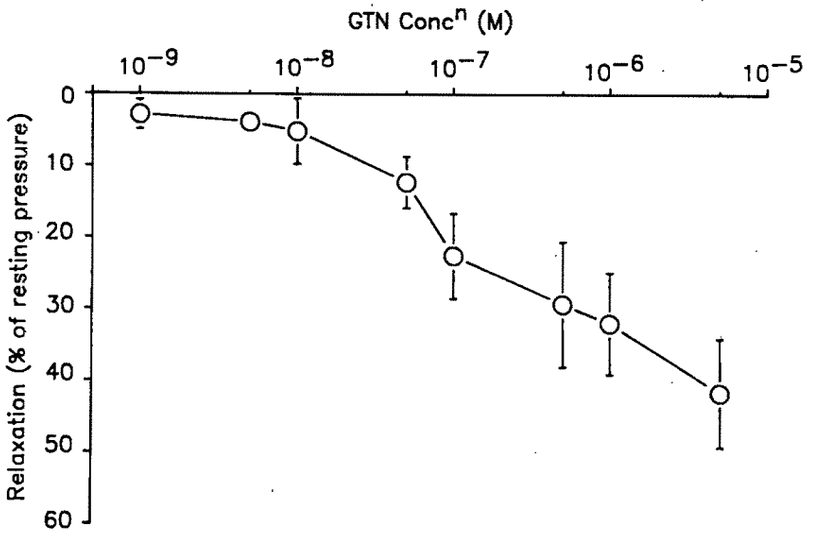


Fig. 2. Effect of glyceryltrinitrate *(GTN)* in U46619-induced vasoconstriction in the fetal-placental circulation. Fetal-placental circulation was preconstricted with U46619 (1 to 5 × 10^{-8} mol/L) and effect of bolus infusion of glyceryl trinitrate (10^{-9} to 5 × 10^{-6} mol/L) on perfusion pressure established. Vasodilation to glyceryl trinitrate is expressed as percentage of relaxation from resting perfusion pressure. Mean ± SEM of four separate experiments is shown. Analysis of variance showed significant concentration-dependent vasodilation ($p < 0.005$) with addition of glyceryl trinitrate.

was allowed before the concentration of glyceryl trinitrate that gave the greatest relaxation was reinjected into the fetal-placental circulation and the response measured. The experiments described with glyceryl trinitrate were subsequently repeated in different placentas using S-nitroso-N-acetylpenicillamine, 10^{-8} to 10^{-4} mol/L, as an alternative agent to generate nitric oxide intracellularly. After establishment of the concentration-dependent relaxation in these experiments, pyrogallol (10^{-4} mol/L) was added to both maternal and fetal perfusates to generate superoxide anion. After a 20-minute equilibration with pyrogallol the concentration of S-nitroso-N-acetylpenicillamine found to give the greatest relaxation was reinjected and response measured.

All experiments were repeated in at least four separate placentas. U46619 was kept as a 10^{-3} mol/L stock in ethanol at −20° C and diluted before use; glyceryl trinitrate was kept as an aqueous solution at 10^{-3} mol/L at 4° C; and S-nitroso-N-acetylpenicillamine was stored in a desiccator at 4° C, solubilized in water before use, kept on ice, and used within 4 hours of preparation. The vasodilator response to glyceryl trinitrate or S-nitroso-N-acetylpenicillamine was expressed as percentage of relaxation from the resting perfusion pressure in each placenta. Analysis of variance with repeated measures was used to determine the significance in change in perfusion pressure on infusion of increasing concentrations of glyceryl trinitrate or S-nitroso-N-acetylpenicillamine. The differences in perfusion pressure in relation to flow in the absence or presence of methylene blue were analyzed by two-way analysis of variance with repeated measures over two variables (flow and the presence or absence of methylene blue). All procedures were approved by the University of Cincinnati College of Medicine Institutional Review Board.

Results

The relationship of perfusion pressure to flow in the fetal-placental circulation is shown in Fig. 1. At all flow rates except 1 ml/min, the perfusion pressure was significantly greater ($p < 0.005$) in the presence of methylene blue (10^{-5} mol/L) than that measured in the absence of methylene blue. The perfusion pressures at each flow rate were significantly different from each other ($p < 0.005$) in both the presence or absence of methylene blue. The increase in perfusion pressure on addition of methylene blue was rapid in onset (<5 minutes, data not shown). However, reversion to media not containing methylene blue was not accompanied by a reduction in perfusion pressure (i.e., the effect of methylene blue appeared irreversible).

Addition of U46619 (1 to 5 × 10^{-8} mol/L) increased perfusion pressure of the fetal-placental circulation. The resulting average resting pressure in the presence of U46619 before addition of glyceryl trinitrate or S-nitroso-N-acetylpenicillamine was 113 ± 12 mm Hg (mean ± SEM, $n = 11$). Bolus infusion of glyceryl trinitrate (10^{-9} to 5 × 10^{-6} mol/L) produced a significant ($p < 0.005$) concentration-dependent vasodilation of the U46619-constricted fetal-placental circulation (Fig. 2). The maximal vasodilation induced by

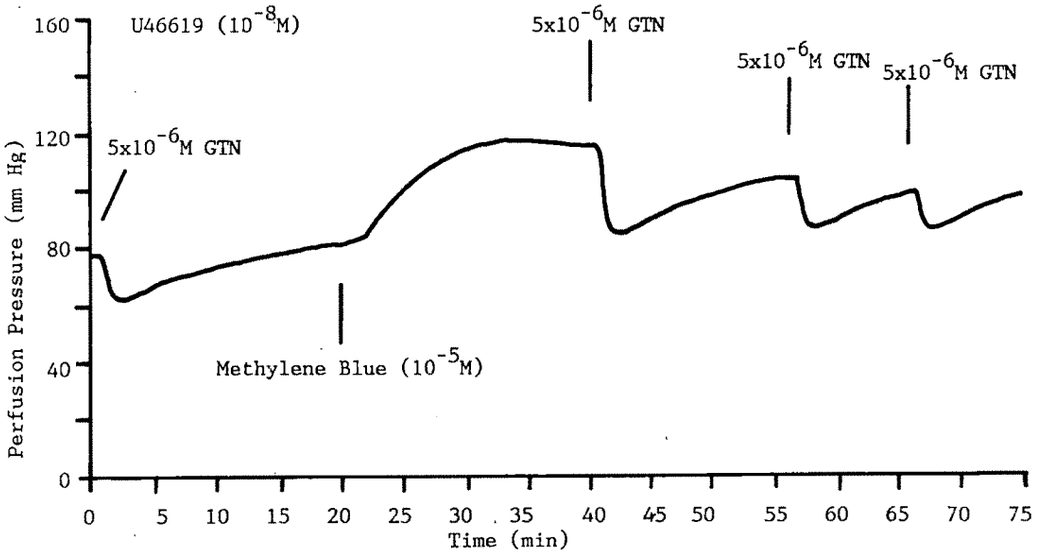


Fig. 3. Effect of methylene blue (10^{-5} mol/L) on glyceryl trinitrate–induced vasodilation. Vasodilator response to glyceryl trinitrate (5×10^{-6} mol/L) in fetal-placental circulation preconstricted with U46619 (10^{-8} mol/L) is shown in representative experiment before and after addition of methylene blue (10^{-5} mol/L) to perfusion medium. U44619 is present throughout.

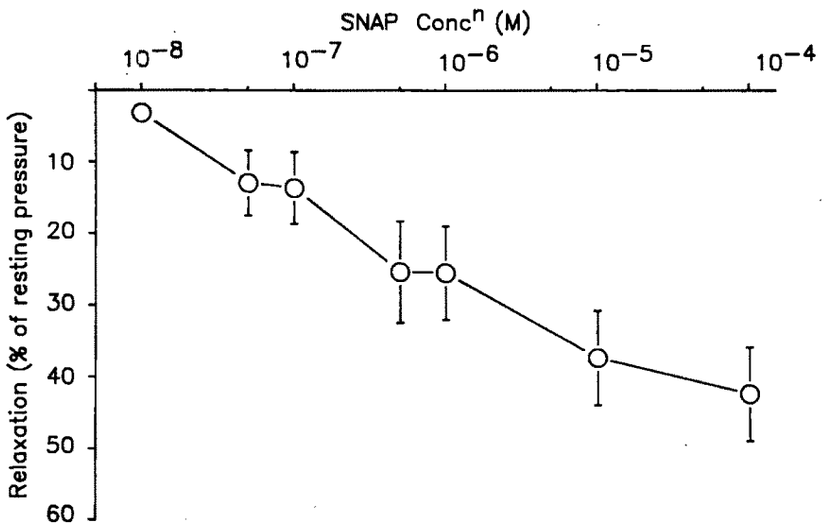


Fig. 4. Effect of S-nitroso-N-acetylpenicillamine *(SNAP)* on U46619-induced vasoconstriction in fetal-placental circulation. Fetal-placental circulation was preconstricted with U46619 (1 to 5×10^{-8} mol/L) and effect of bolus infusion of S-nitroso-N-acetylpenicillamine (10^{-8} to 10^{-4} mol/L) on perfusion pressure measured. Vasodilation to S-nitroso-N-acetylpenicillamine is shown as percentage of relaxation from resting perfusion pressure. Mean ± SEM of seven separate experiments is shown. Analysis of variance showed significant concentration-dependent vasodilation ($p < 0.005$) with addition of S-nitroso-N-acetylpenicillamine.

glyceryl trinitrate gave a decrease in perfusion pressure of 58 ± 19 mm Hg. After infusion of methylene blue (10^{-5} mol/L) into the fetal-placental circulation (in the continued presence of U46619) the subsequent response to glyceryl trinitrate was reduced, compared with the response before methylene blue (Fig. 3). However, the glyceryl trinitrate–induced vasodilation could not be completely abolished by methylene blue, even with the use of a higher concentration of 10^{-4} mol/L methylene blue (data not shown). Although the vaso-

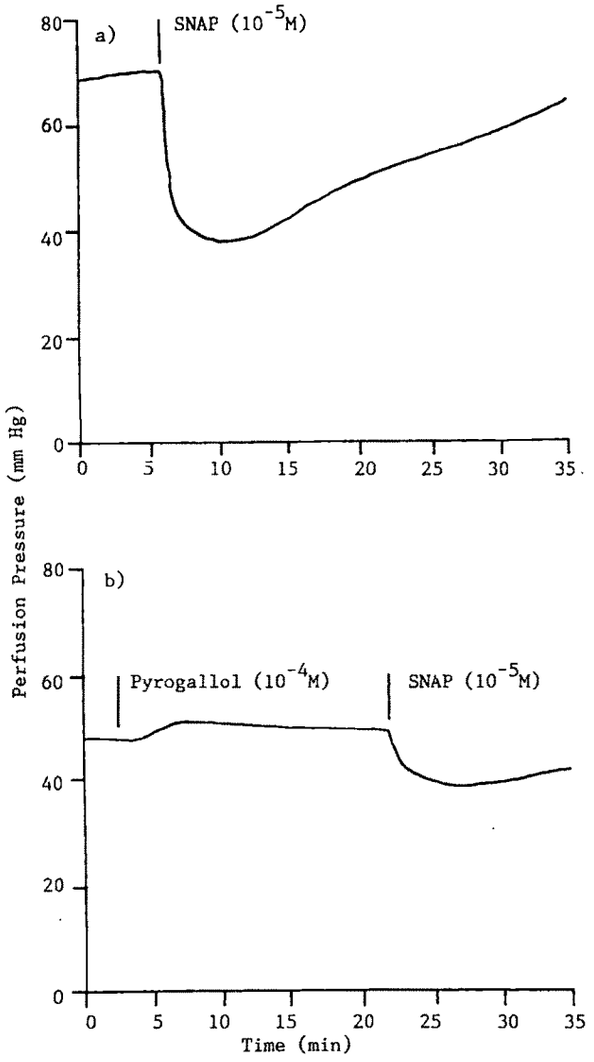


Fig. 5. Effect of pyrogallol (10^{-4} mol/L) on S-nitroso-N-acetylpenicillamine–induced vasodilation. Vasodilator response to S-nitroso-N-acetylpenicillamine (10^{-5} mol/L) in fetal-placental circulation preconstricted with U46619 (10^{-8} mol/L) is shown in representative experiment before (**a**) and after (**b**) addition of pyrogallol (10^{-4} mol/L) to perfusion medium. U46619 is present throughout.

dilator response was not abolished, it was seen to diminish with repetitive infusion of glyceryl trinitrate. The vasodilator response to 5×10^{-6} mol/L glyceryl trinitrate was 60 ± 11, 38 ± 11, and 22 ± 11 mm Hg for the first, second, and third infusions, respectively, after addition of methylene blue (n = 4 experiments). Control experiments (with the absence of methylene blue) showed a constant vasodilator response to repetitive infusion of glyceryl trinitrate, indicating that the diminishing response to glyceryl trinitrate in the presence of methylene blue was not a result of tachyphylaxis.

U46619-induced vasoconstriction of the fetal-placental circulation could also be significantly attenuated ($p < 0.005$) in a concentration-dependent manner by S-nitroso-N-acetylpenicillamine (10^{-8} to 10^{-4} mol/L, Fig. 4). Again, the relaxant effect of S-nitroso-N-acetylpenicillamine could be partially, but not completely, abolished by methylene blue (data not shown) or by perfusion of the fetal-placental circulation (Fig. 5) with pyrogallol (10^{-4} mol/L), which generates superoxide anion and thereby inactivates nitric oxide. The relaxation to 10^{-5} S-nitroso-N-acetylpenicillamine was 42.7 ± 13.1 and 19.8 ± 5.0 mm Hg before and after addition of 10^{-4} mol/L pyrogallol, respectively (n = 5 experiments). Neither glyceryl trinitrate nor S-nitroso-N-acetylpenicillamine was able to significantly relax the fetal-placental circulation perfused at 4 ml/min unless it had been previously constricted with U46619.

Comment

A wide variety of blood vessels have been studied for their ability to exhibit endothelium-dependent relaxation.[2] However, the vessels of the human fetal-placental circulation have not been rigorously investigated. Van de Voorde et al.[7] described the release of endothelium-derived relaxing factor from human umbilical blood vessels stimulated with histamine but did not show an action of that endothelium-derived relaxing factor on the umbilical vessels themselves. The human placental chorionic plate arteries were shown by Griggs and McLaughlin[9] not to relax to either A23187 or histamine, which stimulate endothelium-derived relaxing factor release from umbilical vessels, leading to the conclusion that these arteries had little ability to release endothelium-derived relaxing factor. However, these researchers did show that nitric oxide and sodium nitroprusside were able to relax the chorionic plate artery, findings confirmed by Chaudhuri et al.,[8] who showed relaxation of precontracted human umbilical or placental arteries by glyceryl trinitrate or S-nitroso-N-acetylpenicillamine, both of which generate nitric oxide intracellularly.

We have studied the action of nitric oxide in the placental villus vascular bed, which presumably is a major determinant of resistance in the fetal-placental circulation. It is clear that both glyceryl trinitrate and S-nitroso-N-acetylpenicillamine, which generate nitric oxide intracellularly,[10] are able to relax this vascular bed in a concentration-dependent manner when it has been preconstricted with the thromboxane mimetic U46619. The conclusion that these effects are mediated by nitric oxide is strengthened by our observations that methylene blue antagonizes the vasodilator effect. Methylene blue is an inhibitor of guanylate cyclase activation by nitric oxide.[11] We were unable to totally abolish the effect of glyceryl trinitrate or S-nitroso-N-

acetylpenicillamine even by increasing methylene blue concentrations to 10^{-4} mol/L. This is similar to the observations in other systems.[15] However, we did see a diminution of vasodilator response with increasing time of exposure to methylene blue that did not appear to be a result of tachyphylaxis. Similarly, pyrogallol, which generates superoxide anion and inactivates nitric oxide, was able to prevent the relaxant effects of glyceryl trinitrate or S-nitroso-N-acetylpenicillamine. Reduced hemoglobin acts as an inhibitor of nitric oxide released extracellularly by binding to it to form a nitrosylhemoprotein complex that cannot penetrate the cell membrane. In our hands, reduced hemoglobin (10^{-5} mol/L) infused into the fetal placental circulation did not affect the pressure-flow relationship (data not shown). Thus it appears that nitric oxide is not released extracellularly before action in the fetal-placental circulation.

In the absence of autonomic innervation the fetal circulation of the human placenta may be regulated by autocrine mechanisms, such as flow-induced vasodilation, or by humoral vasoactive agents. However, the mechanisms mediating these mechanisms have not been identified. We previously established the relationship of perfusate flow rate to perfusion pressure, and hence vascular resistance, in the perfused cotyledon and found that blockade of prostaglandin synthesis by meclofenamate had no effect on the flow-pressure relationship.[13] This suggested that a prostaglandin was not involved as a humoral vasodilator. We showed that perfusion of the isolated cotyledon with methylene blue (10^{-5} mol/L) was able to alter the perfusion flow-pressure relationship so that pressure and hence resistance was greater at all flow rates in the presence of methylene blue. This suggests that basal release of nitric oxide may contribute to maintenance of vascular tone in this circulation. In preliminary experiments with the use of a series of compounds that stimulate endothelium-derived relaxing factor release in other vascular beds, we were unable to show vasodilation of the fetal-placental circulation. The stimulus for the release of nitric oxide (i.e., endothelium-derived relaxing factor) in the perfused fetal-placental circulation may be flow or shear stress over the surface of endothelial cells. Increased release of endothelium-derived relaxing factor with increasing rate of flow (continuous or pulsatile) has been shown in canine femoral arteries.[14] The rapidity of the increase in resistance on addition of methylene blue to the fetal-placental circulation indicates that there may be basal release of nitric oxide, which may regulate vascular tone.

In summary, we showed that the fetal-placental circulation has the capacity to generate nitric oxide intracellularly and that nitric oxide can act as a vasodilator. There appears to be basal release of nitric oxide that may contribute to vascular resistance in this circulation.

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Effects of acute hypermagnesemia on the threshold for lidocaine-induced seizures in the rat

Won W. Choi, MD, David S. Warner, MD, Daniel J. Monahan, MD, and Michael M. Todd, MD

Iowa City, Iowa

The effects of acute changes in plasma magnesium concentration on the threshold for lidocaine-induced seizures were evaluated in mechanically ventilated rats receiving 70% nitrous oxide and 30% oxygen. In experiment 1, male rats were intravenously administered either 0.9% sodium chloride (group I) or 5.0% magnesium sulfate to elevate plasma magnesium levels to 5.8 ± 0.1 (group II) or 10.5 ± 1.0 mg/dl (group III). In experiment 2, pregnant rats were intravenously administered either 0.9% sodium chloride (normomagnesemia) or magnesium sulfate, resulting in a plasma magnesium concentration of 7.8 ± 1.4 mg/dl. Thirty minutes later, a continuous intravenous infusion of lidocaine (2.3 mg/kg per minute) was begun in both experiments. Biparietal electroencephalographic activity was monitored continuously. At the onset of electroencephalographic seizure activity, arterial plasma magnesium and lidocaine concentrations were measured. In groups I and III (experiment 1), brain parenchymal magnesium was also assayed. There were no differences in plasma lidocaine concentrations (in experiments 1 or 2) between saline solution and hypermagnesemic groups at onset of seizures. Brain magnesium level was unaltered by magnesium sulfate infusion. We conclude that acute administration of magnesium sulfate alters neither brain magnesium level nor the plasma lidocaine concentration associated with onset of electroencephalographic seizures. (Am J Obstet Gynecol 1991;164:693-7.)

Key words: Anesthetic agents, lidocaine, brain seizures, electrolytes, magnesium, hypermagnesemia

Despite controversy regarding its mode of action,[1,2] magnesium sulfate has long been used for both the prevention and treatment of complications associated with pregnancy-induced hypertension.[3,4] In particular, magnesium sulfate is believed to play a therapeutic role in minimizing convulsant manifestations of this disorder. At the same time, patients treated with magnesium sulfate frequently receive large doses of local anesthetic agents to provide analgesia during obstetric procedures.[5,6] Despite this practice, little information is available about potential interactions between these drugs with respect to central nervous system or cardiovascular toxicity.[7] Thus this study was designed to examine the effects of acute intravenous administration of magnesium sulfate on the plasma lidocaine concentration necessary to induce electroencephalographic seizures in the rat.

From the Department of Anesthesia, University of Iowa College of Medicine.
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Material and methods

Surgical preparation. With institutional Animal Care and Use Committee approved, 39 Sprague-Dawley rats (Amitech, Omaha, Neb.) (weight range, 300 to 450 gm) were used in these experiments. In all animals the surgical preparation was identical. Anesthesia was induced with 4% halothane and maintained with 0.8% halothane in 70% nitrous oxide in oxygen. A tracheostomy was performed and mechanical ventilation adjusted to maintain $PaCO_2$ between 38 to 40 mm Hg and PaO_2 between 80 to 120 mm Hg. A femoral artery was cannulated by means of surgical incision for monitoring arterial blood pressure and blood sampling, whereas the femoral veins were cannulated bilaterally for fluid and drug administration. *d*-Tubocurarine (0.6 mg) and heparin (50 IU) were administered intravenously. Needle electrodes were percutaneously inserted between the temporalis muscle and parietal bones bilaterally, and a reference electrode was placed in the nape of the neck to continuously monitor bipolar electroencephalographic activity (Grass Model 79D, Quincy, Mass.). Rectal temperature was controlled between 36.5° C and 37.5° C by surface heating or cooling. A 45-minute interval was allowed for surgical preparation. All wounds were infiltrated with 1.0% lidocaine, the halo-

thane was then discontinued and the lungs thereafter ventilated with 70% nitrous oxide in oxygen. Given these conditions, a 15-minute stabilization period was allowed before the following procedures.

Experimental design and protocol

Experiment 1. Male rats were allocated randomly to one of three groups. Group I ($n = 8$) received 0.9% sodium chloride intravenously (2 ml/hr). Group II ($n = 10$) received magnesium sulfate (5.0% dissolved in sterile water) intravenously to produce a plasma magnesium level of 5.0 to 7.0 mg/dl. This was achieved by bolus administration of magnesium sulfate (60 mg, 1.2 ml) over 2 minutes, followed by a continuous infusion of 2 ml/hr until termination of the experiment. Group III ($n = 7$) received magnesium sulfate (120 mg, 2.4 ml) over 2 minutes, followed by 100 mg/hr intravenously to produce a plasma magnesium concentration of 9.0 to 12.0 mg/dl. Thirty minutes after onset of the saline solution or magnesium sulfate infusions, a continuous intravenous infusion of 1.0% lidocaine (Astra, Westborough, Maine) in 0.9% sodium chloride was begun at a rate of 2.3 mg/kg per minute. The electroencephalogram was monitored continuously during this time. At the onset of electroencephalographic seizure activity, samples of arterial blood were taken and centrifuged to separate the plasma. Mean arterial pressure (MAP), arterial blood gas pH values, and rectal temperature were also recorded at this electroencephalographic end point. Plasma lidocaine concentration was measured by radioimmunoassay (lidocaine assay, Syva Co., Palo Alto, Cal.). Plasma magnesium concentration was measured by an automated chemistry analyzer (calmagite dye method).

In some rats (group I, $n = 5$; group III, $n = 5$), a thoracotomy was performed immediately after onset of seizures. Via the left cardiac ventricle, the brain was rinsed in situ with 0.9% sodium chloride for 1 to 2 minutes to minimize contamination from blood magnesium. The brains were then removed intact and fast frozen in *n*-pentane (−70° C). Brain parenchymal magnesium concentration was later analyzed by atomic absorption spectroscopy with the use of forebrain tissue for each rat.

Experiment 2. Pregnant rats (14 days' gestation) were subjected to a protocol similar to experiment 1. Rats were randomly assigned to one of two groups. Eight rats received 0.9% sodium chloride intravenously (100 mg/hr), whereas 6 rats were administered a 60 mg bolus of magnesium sulfate over 2 minutes followed by a 100 mg/hr intravenous infusion of 5.0% magnesium sulfate to produce a plasma magnesium concentration of 7.0 to 8.0 mg/dl. Thirty minutes after onset of the saline solution or magnesium sulfate infusion, a continuous intravenous infusion of lidocaine (2.3 mg/kg per minute) was begun. At the onset of electroencephalographic seizure activity, arterial blood was withdrawn for assay of plasma lidocaine and magnesium concentrations. MAP, arterial blood gas pH values, and rectal temperature were also recorded at the onset of seizures.

Data analysis. Because of lack of concurrence, results from the two experiments were evaluated separately. Data were analyzed by a one-way analysis of variance. Posthoc testing was performed to identify between-group differences (experiment 1) when indicated, by a significant F ratio with the use of the Newman-Keuls test. Values are expressed as mean ± SEM. Significance was assumed at $p < 0.05$.

Results

Experiment 1. Before lidocaine infusion, the three groups did not differ in weight, $PaCO_2$, PaO_2, pH, temperature, or MAP (Table I). The magnesium sulfate administered intravenously to groups II and III resulted in plasma magnesium levels of 5.8 ± 0.7 and 10.5 ± 1.0 mg/dl, respectively (Table II). Plasma lidocaine concentrations did not differ between groups at the onset of seizures, nor was there a difference in the total dose of lidocaine administered (Table II). Brain parenchymal magnesium concentrations were similar between groups I (720 ± 14 μg/gm, dry weight) and III (725 ± 12 μg/gm, dry weight) despite the mean plasma magnesium concentration being five times greater in group III (Fig. 1). MAP was comparable between groups before the lidocaine infusion, but was significantly decreased in group III at the onset of seizures, when compared with group I (Table II).

Experiment 2. The normomagnesemic and hypermagnesemic groups did not differ in weight, $PaCO_2$, PaO_2, pH, temperature, or MAP immediately before lidocaine infusion (Table I). Plasma magnesium concentration at the onset of seizures was increased in those animals receiving magnesium sulfate infusion (7.8 ± 1.4 mg/dl) when compared with those that received saline solution (2.0 ± 0.1 mg/dl). In contrast, plasma lidocaine concentrations did not differ between the two groups at the onset of seizures (Table III). At the same time, MAP was significantly decreased in the hypermagnesemic versus normomagnesemic rats (Table III).

Comment

Because the administration of magnesium sulfate is often considered a therapy for convulsive manifestations associated with pregnancy-induced hypertension, we hypothesized that it might also be associated with an increased tolerance of the central nervous system to lidocaine toxicity. Our initial experimental approach involved the use of male rats wherein two levels of hypermagnesemia were induced 30 minutes before intravenous infusion of lidocaine. In this case, the plasma

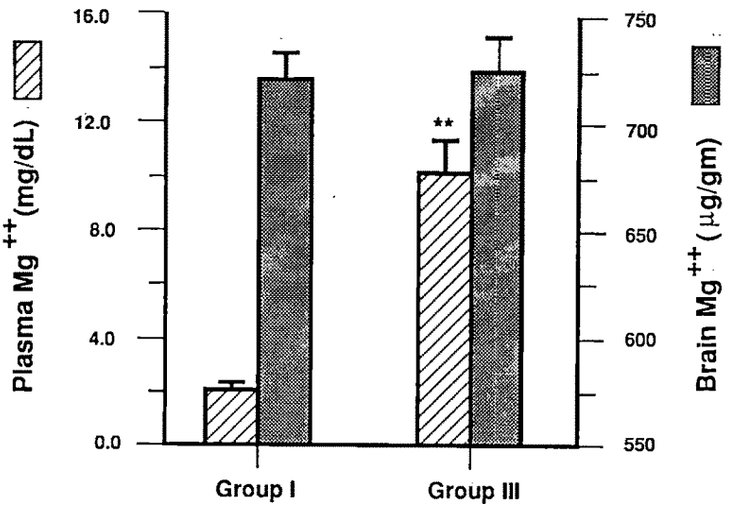


Fig. 1. Arterial plasma and brain parenchymal (dry weight) magnesium concentrations in normomagnesemic (group I) and hypermagnesemic (group III) male rats at the onset of electroencephalographic seizures (experiment 1).

Table I. Physiologic values (mean ± SEM) before infusion of magnesium sulfate or lidocaine

Infusion group	*No.*	*Weight (gm)*	*$PaCO_2$ (mm Hg)*	*PaO_2 (mm Hg)*	*Arterial pH*
Experiment 1 (male rats)					
I	8	419 ± 14	36.6 ± 1.0	91 ± 2	7.45 ± 0.02
II	10	423 ± 14	37.1 ± 0.8	99 ± 4	7.43 ± 0.01
III	7	389 ± 19	38.0 ± 0.3	100 ± 5	7.43 ± 0.01
Experiment 2 (pregnant rats)					
0.9% Sodium chloride	8	358 ± 17	38.0 ± 0.5	112 ± 4	7.41 ± 0.01
5.0% Magnesium sulfate	6	367 ± 36	36.7 ± 0.5	114 ± 6	7.41 ± 0.01

Table II. Arterial plasma magnesium and lidocaine concentrations, MAP, and total dose of lidocaine administered at the onset of electroencephalographic seizures in male rats (experiment 1)

Group	*Magnesium (mg/dl)*	*Lidocaine (μg/ml)*	*MAP (mm Hg)*	*Lidocaine dose (mg/kg)*
I	2.0 ± 0.1	19.2 ± 1.6	109 ± 6	31.1 ± 5.2
II	5.8 ± 0.1*	22.7 ± 2.4	91 ± 4	36.9 ± 5.3
III	10.5 ± 1.0†	20.7 ± 2.8	76 ± 10‡	27.3 ± 3.6

Values are means ± SEM.
*$p < 0.01$ group II versus groups I and III.
†$p < 0.01$ group III versus groups I and II.
‡$p < 0.05$ group III versus group I.

lidocaine concentrations associated with onset of electroencephalographic seizures were found to be similar to those previously reported for both rats and primates,[8, 9] but remained independent of plasma magnesium levels. Corresponding to this, brain parenchymal magnesium concentrations were similar between groups despite a fivefold increase in plasma magnesium level. We then proceeded to determine whether characteristics unique to pregnancy in the rat would produce a different result. Again the plasma lidocaine concentration present at the onset of seizures was unaffected by acute hypermagnesemia, indicating no anticonvulsant activity for this ion. Of note, however, in both male and pregnant rats, the presence of hyper-

Table III. Arterial plasma magnesium and lidocaine concentrations, MAP, and total lidocaine dose administered at the onset of electroencephalographic seizures in pregnant rats (experiment 2)

Group	*Magnesium (mg/dl)*	*Lidocaine (μg/ml)*	*MAP (mm Hg)*	*Lidocaine dose (mg/kg)*
0.9% Sodium chloride	2.0 ± 0.1	28.0 ± 3.0	99 ± 5	31.7 ± 4.0
5.0% Magnesium sulfate	7.8 ± 1.4*	28.0 ± 2.6	56 ± 5*	25.5 ± 3.9

*$p < 0.01$.

magnesemia was associated with significantly lower mean arterial blood pressures at the onset of electroconvulsive activity.

The magnesium ion is a ubiquitous regulator in the nervous system. Specifically, an anticonvulsant activity might be attributable to inhibition of excitatory afferents which use glutamate as a neurotransmitter,[10] where magnesium has been shown to be a noncompetitive antagonist at the postsynaptic conductance channels.[11] Nevertheless, in numerous other well-controlled models of laboratory-induced epilepsy, including bupivacaine hydrochloride toxicity[7] and penicillin-induced seizure foci (pretreatment or post treatment),[12, 13] acute infusions of magnesium sulfate have consistently failed to exhibit anticonvulsant activities.

Other work[14, 15] showed that the bioavailability of magnesium to the brain is severely limited, most likely by the blood-brain barrier. It therefore seems likely that the duration of hypermagnesemia (30 to 45 minutes) in our study was insufficient to allow substantial transport of magnesium to the brain parenchyma. This is consistent with results from other studies evaluating transfer of magnesium from blood to brain. For example, Opelt et al.[16] observed only a 21% increase in cerebrospinal fluid magnesium concentration in dogs over 5.5 hours, while plasma magnesium was held 4 to 5 times normal. In contrast, Chutkow[17] found an increase in cerebral spinal fluid magnesium level identifiable within minutes after an intraperitoneal injection of magnesium chloride into hypomagnesemic rats; however, the cerebral spinal fluid was found not to reflect the brain parenchyma, which lagged several hours behind in showing an increase in magnesium concentration. Given this information, Thurnau et al.[18] evaluated cerebral spinal fluid magnesium levels in parturient preeclamptic women after 4.5 or more hours of magnesium sulfate infusion and found a 19% increase in cerebral spinal fluid magnesium concentration as a result of that therapy.

Thus if magnesium sulfate were to act as an anticonvulsant, it appears as if at least several hours of hypermagnesemia would be necessary to effect events at the neuronal level. Consequently, laboratory investigations, including ours, that have used brief exposures to hypermagnesemia do not rule out the potential for this ion to exhibit anticonvulsant properties against local anesthetic toxicity under conditions of prolonged therapy. In contrast, to the extent that the blood-brain barrier is normal in patients with pregnancy-induced hypertension (a matter that remains under investigation[19]), acute administration of magnesium sulfate should not be expected to protect against local anesthetic neurotoxicity. However, on the basis of the above cited information, further investigation with chronic models of hypermagnesemia or with models that invoke a disrupted blood-brain barrier may yield different conclusions.

A final methodologic consideration requires attention, that being the use of nitrous oxide in our preparation. Nitrous oxide analgesia has been shown to elevate the lidocaine seizure threshold by about 50% in the cat.[20] However, because hypercapnia (which would be expected to accompany large systemic doses of lidocaine) has also been shown to alter lidocaine seizure thresholds,[21] we elected to create an environment where artificial ventilation would be humane and allow control of the background anesthetic and ventilatory state across all groups considered. In fact, during pilot studies in which halothane was continued throughout the lidocaine infusion, we were unable to elicit any convulsive manifestations of lidocaine toxicity. Therefore the background anesthetic, although constant across all groups, is relevant in the interpretation of these results.

In conclusion, when either male or pregnant female rats were rendered acutely hypermagnesemic by an intravenous infusion of magnesium sulfate, the neurotoxic arterial plasma lidocaine concentration was not altered when compared with that observed in normomagnesemic controls. When brain magnesium concentration was quantified, no increases resulting from a fivefold increase in plasma magnesium levels were observed. This data is consistent with the belief that acute administration of magnesium sulfate will not serve to increase the threshold for lidocaine-induced seizures.

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LETTERS TO THE EDITORS

Effect of subject height on the result for the fifty-gram oral glucose tolerance test

To the Editors: Over a 12-month period, 1099 unfasted women at 24 to 28 weeks[3] gestation were screened for gestational diabetes with the 50 gm oral glucose tolerance test.[1] All women with a venous blood glucose value >6.7 mmol/L at 1 hour were retested the following week with the standard 100 gm 3-hour oral glucose tolerance test. The proportion of positive screen results was 9.3%, whereas the prevalence of confirmed gestational diabetes was 1.1%.

Logistic regression analysis established that age, short stature, elevated pregnancy weight, and Asian race are all significant predictors of positive screen results (Table I). Parity, past history of miscarriage, and abortion were other factors assessed but found not significant. Selection of only the data of white women for logistic regression reaffirmed that age and short stature are significant variables associated with risk of positive screen results, whereas weight is only significant in the model when both age and height are included. The mean height and weight of the Asian group increase the relative odds of positive screen results by 25% in comparison with white women. This result cannot be attributed to excess weight because the Asian women have a significantly lower body mass index than that of white women (23.6 ± 3.1, $n = 176$ vs 26.5 ± 4.7, $n = 799$; $p < 0.001$). The body surface area for Asians is also significantly lower (1.54 ± 0.12 vs 1.75 ± 0.16; $p < 0.001$).

Whereas age[2] and Asian race[3] have been reported as important determinants of glucose intolerance, the effect of height and weight has not been cited. In the model developed (Table I) these variables are compensatory, with maximum and minimum effects for short-heavy and tall-light women, respectively. Because body surface area is closely related to height, the present findings imply that the 1-hour blood glucose level after the 50 gm glucose challenge may reflect both glucose mass use as well as glucose intolerance. In support of this statement it has been advocated that the oral glucose tolerance test be adjusted for body surface area.[4]

Table I. Maximum likelihood estimates of logistic parameters relating specific variables to the risk of positive screen test results in pregnant women ($n = 975$)

Variable	*Coefficient*	*SE*	*Probability*
Intercept	7.5609	—	—
Race*	0.66711	0.30491	0.03
Age†	0.55546	0.13506	0.0001
Height (cm)	−0.083238	0.00203	0.0001
Weight (kg)	0.03139	0.00936	0.001

*White = 0; Asian = 1.

†<20 yr = 0; 20 to 24 yr = 1; 25 to 29 yr = 2; >29 yr = 3.

Administration of the glucose load in the unfasted state may also accentuate a dose-related effect.[5]

The overall importance of a dose-effect for the 50 gm oral glucose tolerance test is difficult to judge but highlights a potential source of test variation that is unrelated to glucose intolerance yet may still significantly influence the final result.

G. Phillipou, PhD

Endocrine and Diabetes Laboratory, The Queen Elizabeth Hospital, Woodville, South Australia 5011

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Methotrexate treatment of ectopic gestation and reproductive outcome

To the Editors: I read with interest the report by Stovall et al. (Stovall TG, Ling FW, Buster JE. Reproductive performance after methotrexate treatment of ectopic pregnancy. AM J OBSTET GYNECOL 1990;162:1620-4). There seems to be no doubt that medical treatment of early unruptured ectopic pregnancy offers an attractive alternative to both the patient and the clinician.

However, I am skeptical about whether methotrexate as a folic acid antagonist should be the preferable substance, especially in view of possible teratogenic or mutagenic effects. The authors stated in their report that congenital malformations in subsequent pregnancies seem to be unlikely. The data about the overall teratogenic effect in patients who have had previous exposure to methotrexate, however, are still not satisfactorily clarified. The available figures are based on treatment of gestational trophoblastic neoplasms, and therefore only small numbers of subjects have been analyzed. Although van Thiel et al.[1] indicated no increase in fetal wastage or congenital abnormalities in a series of 50 women, Ross[2] described a 16% spontaneous abortion rate and a 3% stillbirth rate in 58 women, and Walden and Bagshawe[3] reported a total of seven cases (4.4%) with major fetal abnormalities in a group of 159 women who had been previously treated for trophoblastic tumors. These data are derived from women

who were treated systemically, and for the most part, they conceived years after termination of methotrexate therapy.

Furthermore, methotrexate has been shown to be retained in the tissue of men for up to 8 months (probably bound to some receptors), and the persistence was not related to the initial dose.[4] Also, concomitant application of citrovorum rescue did not prevent fixation of methotrexate in the tissue of mice.[5] Therefore the recommended 2-month period for use of a contraceptive device to avoid at least direct teratogenic effects seems to be too short a period of time. Moreover, the possibility that recessive mutations can be induced has not been addressed by any of the recent studies. Most mutations are indeed recessive and therefore are not easily detected unless the trait is sex-linked and thus occurs with greater frequency in male offspring. Observation over several generations would be required to answer this question.

The fact that methotrexate provides a greater likelihood for survival than other therapeutic modalities in patients with a potentially lethal disease does not necessarily indicate its use in cases of tubal pregnancy, especially because more harmless substances, including hyperosmolar glucose solutions[6] or prostaglandins,[7] have also proved to be successful.

C. Egarter, MD

I. Department of Obstetrics and Gynecology, University of Vienna, Spitalgasse 23, A-1090 Wien, Austria

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Reply

To the Editors: We thank Dr. Egarter for his interest in our work on methotrexate treatment of ectopic pregnancy and, in particular, his interest in our article. During the last several months much has been written and several different medical alternatives have been described for the treatment of unruptured ectopic pregnancy. These have included systemic methotrexate, laparoscopic or transvaginal injection of methotrexate, potassium chloride, hyperosmolar glucose, prostaglandins, or RU-486. To date, most experience is in the area of systemic methotrexate. We believe that it will be the agent of choice for this condition, especially in light of the nonlaparoscopic diagnostic approach[1] and refinement of the regimen to only a single dose of methotrexate without citrovorum recovery.[2]

Dr. Egarter is correct in his concern that the reproductive effects of methotrexate after treatment of ectopic pregnancy have not been fully established. However, the data to date with respect to gestational trophoblastic disease are reassuring. In addition, the duration of therapy and the total dose of methotrexate given are much less than those in patients with trophoblastic disease and therefore represent another piece of reassuring data. The data of Ross[3] do indeed show a 16% spontaneous abortion rate and a 3% stillbirth rate. He states that his data do not include other known risk factors for fetal wastage, such as maternal age, and therefore the results are speculative. In addition, a 16% spontaneous abortion rate is well within normal limits, depending on the time the gestation is documented. Kjer and Iversen[4] reported no increase in the spontaneous abortion rate after combination chemotherapy with methotrexate and actinomycin D. Hammond et al.[5] reported similar results in 29 pregnancies with no increase in either the spontaneous loss or the congenital anomaly rate. Finally, Rustin et al.[6] showed no increase of second tumors after cytotoxic chemotherapy in 457 long-term survivors who underwent treatment for choriocarcinoma or invasive mole.

No study to date has done a systematic evaluation with a control group to evaluate offspring of patients treated with methotrexate. However, the weight of the evidence would favor that methotrexate is not mutagenic and does not increase the spontaneous abortion or fetal loss rate.

We believe that methotrexate therapy for unruptured ectopic pregnancy will gain wide acceptance as the nonlaparoscopic protocol and the single-dose regimen are considered.

Thomas G. Stovall, MD, Frank W. Ling, MD, and John E. Buster, MD

Department of Obstetrics and Gynecology, University of Tennessee, 853 Jefferson Ave., Room E-102, Memphis, TN 38103

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phoblastic neoplasms. AM J OBSTET GYNECOL 1967;98: 71-8.
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Predictive value: Symptoms of preterm labor

To the Editors: Preterm birth is the greatest single cause of neonatal morbidity and mortality in the United States,[1] and as such deserves a concerted effort directed toward its prevention.The well-known continuing efforts of Drs. Robert Creasy and Michael Katz to sensitize obstetricians to the problem of preterm labor are commendable.

However, their recent article, which describes early signs and symptoms of preterm labor (Katz M, Goodyear K, Creasy RK. Early signs and symptoms of preterm labor. AM J OBSTET GYNECOL 1990;162:1150-3) contains a deceptive probability error and draws conclusions that require disputation.

The probability error is in Table V of their article, the calculation of predictive values. The incidence of preterm birth nationwide in 1980 was 3.55%; among black women the rate was 6.77%.[2] The article does not describe the demographics of the study population, but to derive the values reported in the table the incidence of preterm labor would have to be 50%!

A basic rule of medical probability (epidemiology) is to ask the question: "Is my answer reasonable?" Acceptance of Table V would require one to believe, for instance, that 86% of pregnant patients with urinary frequency are in, or will develop, preterm labor. In my view, this is not a reasonable answer; therefore, the other answers (which were derived by the same methods) must be viewed with circumspection.

If incidence of preterm labor among black women in the United States were used to calculate predictive values, the numbers would appear as in Table I.

These numbers are more reasonable and, on the basis of the findings of the study, are more "computationally" correct. Not surprisingly, the positive predictive values have decreased and the negative predictive values have increased.

Table I. Predictive values of certain signs and symptoms in a population with a 7% incidence of preterm labor

Sign or symptom	*Positive predictive value*	*Negative predictive value*
Contractions	0.52	0.98
Menstrual cramps	0.39	0.96
Intestinal cramps	0.39	0.94
Constant backache	0.65	0.95
Constant pelvic pressure	0.33	0.94
Vaginal discharge, amount	0.30	0.96
Vaginal discharge, color	0.39	0.95
Urinary frequency	0.32	0.94

The findings, however, must still be questioned because of the retrospective design of the study. The extrapolation of data derived from sampling a small portion of that population to the population as a whole is valid only if the selection of the sample population and the collection of data are done without bias. The authors concede that, "... a potentially significant limitation of retrospective questioning as done in this study cannot be ignored." This limitation is called "recall" or "ascertainment" bias and occurs because patients who are hospitalized for preterm labor are more likely to remember antecedent symptoms when the symptoms are associated with a pregnancy complication than are patients without a pregnancy complication who are being questioned in an outpatient setting.

The authors predict candidly, however, that a decline in sensitivity would occur if they relied on prospective, patient-initiated reporting because, "... pregnant women are more likely to be distracted in the out-of-hospital environment." They apparently make this point to emphasize the importance of educational prevention programs, but even an independent reviewer could not have made a statement more damaging to the credibility of this study.

I would petition Drs. Creasy and Katz to continue their superb educational efforts and to refine their meritorious surveillance techniques so that in the near future they may provide us with clinically applicable conclusions that are derived from data that have been obtained in an unbiased, prospective manner.

Daniel M. Strickland, MD
Department of Obstetrics and Gynecology, Dhahran Health Center, Dhahran 31311, Saudi Arabia

Address for correspondence:
PSC Box R-602, APO New York, NY 09616

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Reply

To the Editors: We thank Dr. Strickland for his excellent and insightful critique. Although we agree with many of the comments, we would like to point out a few issues that could help clarify the conclusions in our recent report (Katz M, Goodyear K, Creasy RK. Early signs and symptoms of preterm labor. AM J OBSTET GYNECOL 1990;162:1150-3.). Our Table V indeed gives the values that were computed in a population, which by our choice, had a 50% incidence of preterm labor. These facts are clearly given in our Material and methods section and can therefore hardly be considered to "contain a deceptive probability error." Once it is clear that the data in Table V are relevant for those high-risk populations whose incidence of preterm labor is

50% (multifetal gestations, multiple previous spontaneous preterm births, etc.), the calculations of predictive values are not only valid and reasonable but also "computationally" correct. Needless to say, because of the direct relationship between the incidence of an event and predictive values, a lower incidence of preterm labor will result in lower positive predictive values. This is very eloquently demonstrated in Dr. Strickland's computations for a 7% incidence of preterm labor. Nevertheless, even these "lower" positive predictive values, which range from close to 40% and up to 65% for signs or symptoms such as cramps, change in vaginal discharge, or constant backache, are very impressive and could serve quite well for the *purpose of screening* as to the possibility of preterm labor.

It should be emphasized that we did not, and still do not, propose use of these signs and symptoms to establish the diagnosis of preterm labor, but rather to screen patients' complaints. It is of note that a recent study by Iams et al.[1] confirmed the importance of these signs and symptoms in helping to screen patients who are at increased risk of preterm labor. More recently, Martin et al.[2] reported that among their patients who had preterm labor, subjective symptoms were the sole reason for evaluation in almost one quarter of the patients. Again, as stated in our article, all of the above-mentioned data cannot replace the need for a prospective unbiased study. It is indeed unfortunate that in spite of the fact that preterm delivery is the greatest contributor to perinatal mortality from any complication of pregnancy, that not one word was published on the early signs and symptoms of preterm labor when this study was begun. We eagerly invite others, and Dr. Strickland, to contribute their knowledge on the early signs and symptoms of preterm labor, which is the most significant hurdle to overcome in the achievement of a satisfactory reproductive outcome. However, until such additional data are available, use of such signs and symptoms for screening of reports that are made by high-risk patients, seems prudent.

Dr. Strickland's comments about the retrospective nature of our study and the issues of our subjects' recall bias were extensively stressed previously in our Comment section. We raise these issues not only to point out a potential role for educational prevention programs but also to discuss the potential weaknesses of our study. We thus accept Dr. Strickland's comments about us being our own severest critics with deep appreciation.

Robert K. Creasy, MD, and Michael Katz, MD
Department of Obstetrics and Gynecology, University of Texas Medical School, 6431 Fannin, Suite 3.286, Houston, TX 77030

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Successful treatment of postpartum shock caused by amniotic fluid embolism with cardiopulmonary bypass and pulmonary artery thromboembolectomy

To the Editors: Esposito et al. (Esposito RA, Grossi EA, Coppa G, et al. Successful treatment of postpartum shock caused by amniotic fluid embolism and pulmonary artery thrombolectomy. AM J OBSTET GYNECOL 1990;163:572-4.) are the first to describe thromboembolic elements in the pulmonary arteries in a case of amniotic fluid embolism, but they did not understand the pathogenetic significance of their observation.

It has been shown by Stolte (1971),[1] Lim (1972),[2] and Courtney and Allington (1972)[3] that amniotic fluid has clot-promoting activity. Amniotic fluid is not in need of proconvertin,[2] for it contains a proconvertin-like substance, and in high dilutions it is cephalin dependent.[4]

Animal experiments of Stolte et al.[5] and Lim[6] showed that the effects of amniotic fluid infusions were distinct from the clinical picture of amniotic fluid embolism. Such infusions give rise to a state of hypercoagulability: In the *circulating* blood 1 minute after injection, there was a shortening of the intact and activated clotting time, without a change in the level of factor I and the number of platelets[2] and with a simultaneous increase of factor V activity.[4] In *stagnant* blood, amniotic fluid infusion led to the formation of a clot.[1, 4, 7] Stasis of blood in the inferior vena cava after amniotic fluid infusion led to the formation of a clot, which, once the flow of blood was restored, was displaced to the pulmonary vessels.[4]

Only extremely large amounts of amniotic fluid caused diffuse intravascular clotting.[4] In an analysis of the mode of death in 96 cases of fatal amniotic fluid embolism from the literature, Kool[4] discerned the cases in which cardiovascular, respiratory, and cerebral accidents were responsible for the death from those in which bleeding that resulted from diffuse intravascular clotting led to maternal death. In the latter group rupture of the uterus was significantly more frequent.

From the investigations of Kerr (1964),[8] we know that in pregnant women in the second trimester of pregnancy, there is stasis of blood in the inferior vena cava when they are in the supine position. From this and in the light of the above-mentioned experiments, we believe that after laceration of the uterine blood vessels, amniotic fluid that reaches stagnant pools of blood in the large veins causes formation of clots, which later, when the flow of blood has been restored after delivery, reaches the lungs and causes the cardiorespiratory accident. When the amniotic fluid reaches the maternal circulation via a traumatic area (e.g., a rupture of the uterus) in which large amounts of thromboplastin are available, the amniotic fluid acts as a vehicle of this thromboplastin and causes diffuse intravascular clotting and a fatal loss of blood.

M.J. Kool, MD, PhD
Catharina Ziekenhuis, Michelangelolaan 2, 5602 Za Eindhoven, The Netherlands

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Intradecidual T lymphocytes lack T-cell receptors

To the Editors: We read with great interest the article by King and Loke (King A, Loke AW. Uterine large granular lymphocytes: A possible role in embryonic implantation? AM J OBSTET GYNECOL 1990;162:308-10) and would like to make some additional remarks on this subject.

In a recent report[1] we described the lack of expression of the two variants of the T-cell antigen receptor by mature T lymphocytes in first-trimester decidua that was obtained from women who were undergoing therapeutic abortions. Immunohistochemical analysis revealed considerable numbers of $CD3^+$ cells in the decidua, but antibodies against the structural components of the T-cell antigen receptor stained only very few (α/β–chain) or no (γ/δ–chain) cells. Thus the phenotype of these mature T cells can be described as follows: $CD3^+$, $\alpha/\beta-$, $\gamma/\delta-$. Normal percentages of $\alpha/\beta+$ T cells, however, could be demonstrated in the peripheral blood of these women. After in vitro culture for 3 weeks with phytohemagglutinin-H and recombinant interleukin-2, intradecidual T cells expressed the T-cell antigen receptor. Fluorescence-activated cell sorter analysis revealed $CD3^+$, $\alpha/\beta+$, and $\gamma/\delta-$ T cells. Our data clearly show that mature T cells, which constitute only a minority of the intradecidual leukocytes, do not express immunohistochemically detectable amounts of T-cell antigen receptor during early normal pregnancy. Since it is known that CD3 is not usually expressed on the cell surface without coexpression of the α/β or γ/δ heterodimer,[2] one explanation for our results could be that the density of T-cell antigen receptor proteins is below the threshold for detection with the antibodies that were applied (αF1, βF1, BMA031, WT31, TCR δ_1, δTCS1). This could be due to partial or complete internalization of the T-cell antigen receptor/CD3 complex. It is also possible that a conformational change has rendered the receptor molecule undetectable by the anti–T-cell antigen receptor antibodies. Either of these mechanisms could interfere with T-cell antigen recognition, since high-affinity binding cannot occur under such circumstances. As a consequence, there would be a state of anergy toward fetal antigens. This is probably one of the decisive mechanisms that is involved in local tolerance of the maternal immune system to the embryo.

Johannes Dietl, MD, Hans-Peter Horny, MD, Peter Ruck, MD, Klaus Marzusch, MD, and Edwin Kaiserling, MD

Department of Obstetrics and Gynecology and Department of Pathology, University of Tübingen, Schleichstr. 4, 7400 Tübingen, Federal Republic of Germany

Henrik Griesser, MD

Department of Pathology, University of Kiel, Michaelisstr. 11, 2300 Kiel, Federal Republic of Germany

Dieter Kabelitz, MD

Department of Immunology, University of Heidelberg, Im Neuenheimer Feld 305, 6900 Heidelberg, Federal Republic of Germany

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Response declined

Intraumbilical injection of oxytocin

To the Editors: I read with interest the recent correspondence by Golan (Golan A. Volume and timing are key to use of intraumbilical oxytocin for management of retained placenta. AM J OBSTET GYNECOL 1990;162: 1628-9). Dr. Golan first stated that the *routine* use of intraumbilical oxytocin shortens the third stage of labor. Subsequently, he stated that one should not inject the oxytocin until 30 minutes after delivery. Indeed, he criticized us[1] for injecting the oxytocin too soon (i.e., 5 minutes) after delivery. I am confused. If one is going to use this technique on a routine basis (a practice that I do not recommend), how can one wait until 30 minutes after delivery?

David H. Chestnut, MD

Departments of Anesthesia and Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, IA 52242

REFERENCE

1. Chestnut DH, Wilcox LL. Influence of umbilical vein administration of oxytocin on the third stage of labor: a randomized, double-blind placebo-controlled study. AM J OBSTET GYNECOL 1987;157:160-2.

Reply

To the Editors: I appreciate Dr. Chestnut's comment but regret, however, that I was misunderstood. Neri et al.[1] and, recently, Reddy and Carey[2] have demonstrated the usefulness of the routine use of oxytocin in the management of the third stage of labor. The latter have shown in a randomized, controlled manner that such an application shortens the duration of the third stage and consequently diminishes blood loss.

The unnecessary confusion arises from the lack of differentiation between the *management of the third stage of labor* and the *management of the retained placenta.* I have consistently referred to the latter practice, and in spite of my appreciation of the findings and the clearly presented point by Reddy and Carey,[2] this was never our practice and I have never recommended such routine use of oxytocin for the management of the third stage of labor.

Intraumbilical injection of oxytocin seems to be an effective, quick, and safe method for the separation and delivery of the *retained placenta.* We recommend a waiting period of 30 minutes.[3]

Abraham Golan, MD

Department of Obstetrics and Gynecology, Assaf Harofe Medical Center, P.O.B. Beer Yaakov, Zerifin, 70300, Israel

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Rh immune globulin prophylaxis for threatened abortions

To the Editors: I read with interest the article by Dayton et al. (Dayton VD, Anderson DS, Crosson JT, Cruikshank SH. A case of Rh isoimmunization: should threatened first-trimester abortion be an indication for Rh immune globulin prophylaxis? AM J OBSTET GYNECOL 1990;163:63-64). In this report, a patient with an Rh negative blood type with a first-trimester threatened abortion became isoimmunized to the D antigen later in her pregnancy. The authors make the assumption that the threatened abortion was the cause of the subsequent sensitization.

However, an antibody screen, which was performed 2 weeks after her presentation to the emergency room because of cramping and bleeding, was negative. At the time of a genetic amniocentesis at 15 weeks' gestation, the antibody screen was still negative, and the patient was given 300 μg of Rh immune globulin prophylactically. The next antibody screen at 29 weeks' gestation was positive, the cord blood antiglobulin test was strongly positive, and the patient's antibody screen remained positive even 6 months post partum. The authors make the assumption that the cause of the isoimmunization was the minor first-trimester hemorrhage.

The frequency of transplacental hemorrhage that occurs with threatened abortions is unknown. The use of Rh immune globulin for treatment of threatened abortions is thus not clearly defined. However, several alternative explanations for the patient's isoimmunization exist. First, a large transplacental hemorrhage may have occurred during the amniocentesis, and the standard dose of Rh immune globulin therefore may have been inadequate. The details of the amniocentesis are not described; we do not know the number of attempts at needle placement or whether the needle was directed through an anterior placenta.

Second, the presence of fetal red blood cells in the maternal circulation increases throughout gestation. Cohen et al.[1] and Clayton et al.[2] have shown, with the use of the Kleihauer-Betke test, that significant numbers of patients had fetal red blood cells in the maternal circulation, especially in the second and third trimesters. This significant degree of clinically silent transplacental hemorrhage may help explain the small percentage of women who become isoimmunized before the twenty-eight-week Rh immune globulin injection. A silent transplacental hemorrhage may well have occurred in this patient.

In summary, although the authors raise an important question, "Should Rh immune globulin be administered to Rh negative women with threatened abortions?," they do not sufficiently prove that a relationship existed in their patient between the bleeding that occurred during the first-trimester and the subsequent isoimmunization. What is needed is a study that compares the incidence of transplacental hemorrhage in women who present with threatened abortions versus a first-trimester control population. This study is currently ongoing.

Jeffrey A. Kuller, MD

Department of Obstetrics, Gynecology, and Reproductive Sciences, Reproductive Genetics Unit, University of California, San Francisco, San Francisco, CA 94143-0720.

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Reply

To the Editors: Thank you for forwarding Dr. Kuller's letter to us. His questions allow us to emphasize some very important points that, because of space limitations, we could not more fully illustrate in our report.

First, how could one be sure that the standard 300 μg vial of Rh immune globulin was sufficient to cover any bleeding from the amniocentesis at 15 weeks' gestation? Precise measurement of the volume of the fetoplacental circulation for all times during pregnancy is not available; however, the fetoplacental blood volume at term is approximately 125 ml/kg of fetal weight.[1] According to published tables, at 16 weeks' gestation a fetus weighs approximately 105 gm,[2] and therefore would have a whole blood volume (fetus and placenta) of approximately 13 ml. The standard vial of Rh immune globulin is sufficient to cover an exposure to 30 ml of Rh_o(D)-positive blood.[3] We made the assumption that this would be enough to cover even a lethal transplacental hemorrhage at 15 weeks' gestation

unless there was a product failure of the Rh immune globulin. To the best of our knowledge, there have been no reported failures of the product and lot number that our patient received.

Next, why was the antibody screen for our patient negative at 10 weeks' and at 15 weeks' gestation if the patient presumably was sensitized from a bleeding episode at 8.5 weeks' gestation? This is an extremely important point for clinical obstetricians. First, it requires several weeks for a primary response to a blood-group antibody to be detectable.[3] Second, we reported that the 15-week antibody screen for our patient was only performed with low ionic strength saline solution and without any enzyme enhancement. The anti-D antibody frequently requires enzyme enhancement to be detected if it is weak or developing[3] (as it would be at 15 weeks' gestation in our patient). For this reason, many laboratories routinely use enzyme enhancement for all obstetric antibody screening. We are confident that lack of enzyme enhancement was the reason that the seroconversion was not detected at 15 weeks' gestation in our patient.

As to whether or not this patient could have been sensitized as a result of small transplacental hemorrhages between the fifteenth and twenty-eighth gestational weeks, we again contend that the full dose of Rh immune globulin was more than ample to cover any bleeding from the amniocentesis at 15 weeks' gestation, and sufficient Rh immune globulin should have been present to cover any minor bleeding episodes between that time and 28 weeks' gestation.

Again, we thank you for giving us this opportunity to clarify these important points. We would also again like to question why it is that the American College of Obstetrics and Gynecology recommends the routine use of Rh immune globulin after chorionic villus sampling in the first trimester but not for spontaneous transplacental bleeding during the same time period.[4] Until a study such as the one Dr. Kuller mentions indicates otherwise, we will continue to recommend that our physicians give Rh immune globulin to women with the Rh_o(D)-negative blood type who have first-trimester bleeding whether or not they go on to abort spontaneously.

Vanessa D. Dayton, MD, Deborah S. Anderson, BS, John T. Crosson, MD, and Stephen H. Cruikshank, MD*

Departments of Laboratory Medicine and Pathology and Obstetrics and Gynecology, Hennepin County Medical Center, Minneapolis, MN 55415

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Koch's postulates: Revisited or revised?

To the Editors: During my years as an obstetrician-gynecologist, I have come to view the GRAY JOURNAL as the preeminent publication in our specialty. I have believed that the JOURNAL has set the standard for publications in our specialty. I am sure that our colleagues throughout the world agree.

It is therefore with a sense of dismay that I write this letter to you regarding a recently published article [Wiggins DA, Elliott JP. Oligohydramnios in each sac of a triplet gestation caused by Motrin—Fulfilling *Koch's postulates* (italics added). AM J OBSTET GYNECOL 1990;162:460-1].

It is, perhaps, that as I grow older my natural tendencies as a gadfly become more pronounced or, perhaps, my sense of purism is disturbed by historic inaccuracies, to wit: In 1882, Robert *Koch,* a Nobel Laureate in 1905, formalized the criteria that established the pathogenic character of a microorganism from that of an adventitious microbe. This statement became known as "Koch's postulates" (Koch R. Berl Klin Wochenschr 1882;19:221-30). These formalized criteria, which were introduced by Henle in 1840, state: (1) The organism is regularly found in lesions of the disease; (2) it can be isolated in pure culture on artificial media; (3) inoculation of this culture produces a similar disease in experimental animals; (4) the organism can be recovered from the lesions in these animals.

How the above could be unappreciated by the authors (who did not even present a reference to these "postulates") and how this could be overlooked and accepted by the Editors of the JOURNAL without comment is startling. Indeed, repeatedly the name *Koch* is misspelled as *Kock.*

I do not take offense with the fact that the authors wish to establish an appropriate deductive path to establish their case for the relationship of Motrin to oligohydramnios. However, it would have been preferable for them to use some approach other than the application of a set of principles that relates to microbial pathogenicity.

This editorial oversight is upsetting!

Thank you for patience in reviewing this matter.

Burton A. Krumholz, MD

Professor of Gynecology and Obstetrics, Albert Einstein College of Medicine, Long Island Jewish Medical Center, New Hyde Park, NY 11042

Response declined

Frontothalamic measurement in the second trimester of pregnancy

To the Editors: After we read the report of Goldstein, which was published in the JOURNAL in 1988 (Goldstein I, Reece EA, Pilu G, O'Connor TZ, Lockwood CJ, Hobbins JC. Sonographic assessment of the frontal lobe: A potential tool for prenatal diagnosis of microcephaly. AM J OBSTET GYNECOL 1988;158:1057-62) and which detailed a new fetal brain measurement, the

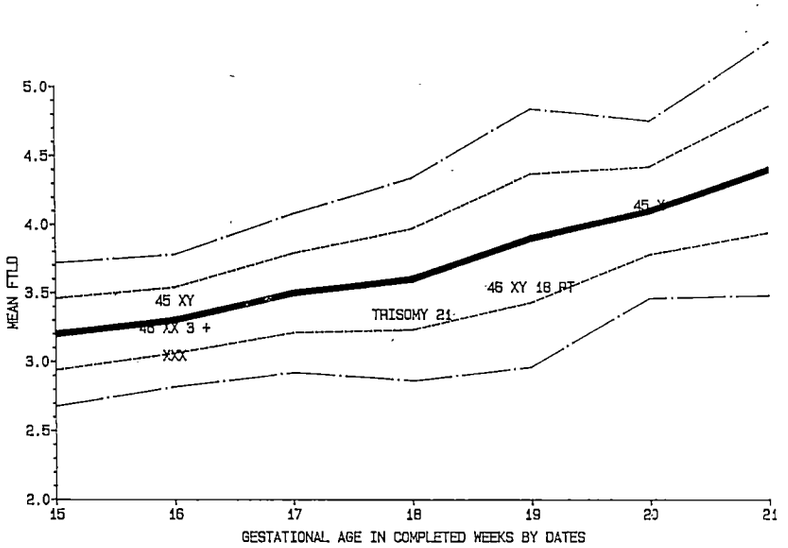


Fig. 1. Frontal thalamic lobe diameter (*FTLD* = 0.865 + BPD × 69.9) as determined by ultrasonography according to gestional age by dates. BPD is biparietal diameter. *FTLD* values are: ▬▬▬, mean; ————, +1 SD; —●—●—●—, ±2 SD.

"frontothalamic distance," we undertook a prospective study to determine whether there was any benefit in incorporating this measurement into routine second-trimester obstetric scanning. Our hope was that chromosomally abnormal fetuses could be distinguished by subtle changes that are due to a tendency toward microcephaly. Three hundred forty-two patients who were scheduled for amniocentesis were prospectively studied. Within this group, six abnormal chromosome analyses were found and plotted against the normal range that was found in our sample population. None of the chromosomally abnormal fetuses (trisomy 21, 46,XY; 45,X; 45,XY; XXX; 46,XX3+) fell outside of 1 SD of our prospective sample (Fig. 1).

In addition, a retrospective review of images that were previously obtained for seven trisomy 21 gestations were matched in a control group of 37 normal ultrasonographic studies. There was no significant difference in the frontothalamic lobe measurements for the trisomy group. Our evaluation suggests that, in spite of the authors' findings of its potential use in the prenatal diagnosis of microcephaly, this particular measurement does not appear to aid in the early diagnosis of chromosomally abnormal fetuses.

Michael Crade, MD, Kathy Crade, and Roger K. Freeman, MD

Long Beach Memorial Medical Center, 2801 Atlantic Ave., Long Beach, CA 90806

Response declined

Prevalence of acquired immunodeficiency syndrome detected during prenatal care of population of low socioeconomic status

To the Editors: With reference to the article "Prevalence of human immunodeficiency virus in a general prenatal population" (Barton et al. AM J OBSTET GYNECOL 1989;160:1316-24) we would like to transmit the experience of the Faculdade de Ciências Médicas de Santos of São Paulo, Brazil. During 1988, we interviewed 803 pregnant women from our prenatal program. Of these, 514 agreed to undergo tests for the detection of anti-human immunodeficiency virus antibodies. We found 27 cases (5.2%) of positive enzyme-linked immunosorbent assay tests of which 19 cases (3.75%) were further confirmed by the synthetic peptide LGIWGCSKLIC test with the use of the gp 41 fraction. Our criteria to characterize the risk factors (Table I) differed from those established by Dr. Barton, probably because of socioeconomic factors and populational habits. We verified that of the 803 pregnant women who

Table I. Risk factors for AIDS to pregnant women

Two or more partners per month
Anal intercourse
Use of intravenous drugs
Sexual intercourse with intravenous drug addicts
Blood transfusions in the last 7 years
Partner with positive ELISA
Initiation of sexual life before the age of 15

ELISA, Enzyme-linked immunosorbent assay.

Table II. Distribution of the 803 pregnant women according to the risk rate for AIDS

Risk rate	*Factors*	*No.*	*%*
Low	0	447	55.6
Medium	1	212	26.4
High	≥2	144	18.0

were interviewed, 26.4% had one risk factor and 18% had two or more risk factors (Table II). Therefore 44.4% of our pregnant women have at least one risk factor for acquired immunodeficiency syndrome (AIDS). The positivity of anti-human immunodeficiency virus in women with no risk factor was 0.3%; in those with one risk factor it rose to 3.6%, and in the group with two or more risk factors it reached 14.1%.

The city of Santos has one of the world's highest prevalence rates for AIDS (61 cases per 100,000 inhabitants). Of the 520 cases that were registered before March 1990, 35% occurred in the female population. Therefore in this city, out of every 2000 women, one has AIDS. These results show the danger of heterosexual transmission and predict the high number of cases of AIDS that we will face in the near future.

Antonio Guilherme Moreira Porto, PhD
Department of Obstetrics, Faculdade de Ciencias Medicas de Santos, Rua Oswaldo Cruz 179, CEP 11.100-Santos, Sao Paulo, Brazil

Reply

To the Editors: I applaud Dr. Antonio Guilherme Moreira Porto's work in São Paulo, Brazil, and find his observation of our work most interesting. Again, however, it points out the confusion we suffer from in comparing data when we are restricted in our ability to use verified, standardized epidemiologic techniques in investigation of this disease.

The following are a few comments concerning the methods and findings in our article versus Dr. Porto's.

We do not consider a single positive enzyme-linked immunosorbent assay to be significant. A repeat procedure is ordered and only if *both* are positive do we submit specimens for more definitive Western blot studies.

The incidence of one of every 2000 women with acquired immunodeficiency syndrome in São Paulo is truly startling. I can only assume there is a high incidence of prostitution and/or intravenous drug use in this community.

It is interesting that the distribution of risk factors by percent (>37%) is much higher than ours. Indeed our principal risk factors are even different.

I submit a brief summary of the Chicago experience with acquired immunodeficiency syndrome to date for the Editors and authors to consider in comparing the Chicago experience with the São Paulo experience.

Since our study was first presented, we have now tested >1600 pregnant women in our clinics and the statistical outcomes have not significantly altered. By far (80%), the infections in our female population have been related to substance abuse by either patient or partner(s). This remains the highest risk factor in our study population.

John J. Barton, MD
Department of Obstetrics and Gynecology, Illinois Masonic Medical Center, 836 West Wellington Ave., Chicago, IL 60657

A chill wind blows fresh air

To the Editors: The Clinical Opinion article by Chavkin and Rosenfield in the August 1990 issue (Chavkin W, Rosenfield A. A chill wind blows: Webster, obstetrics, and the health of women. Am J Obstet Gynecol 1990;163:450-2) demonstrates the seriousness of the threat to abortion on demand that has been posed by recent events. The threat has now mobilized abortion rights supporters to action, which now apparently includes exercising the constitutional right to hyperbole. Chavkin and Rosenfield's arguments include raising the specter of thousands of dead women and a police state with listening devices in examination rooms. In addition, they seem to believe that abortion is a reasonable solution to most adverse perinatal and maternal health outcomes. Their conclusion is presumptuous at best when they imply that truly dedicated obstetricians will work vigorously to ensure that unrestricted abortion is part of reproductive health care.

In maternal-fetal medicine practice I care for two patients in the mother and the unborn child, and I rarely find a medical need to interfere with the continued existence of the fetus. The majority of abortion opponents have no argument with abortion for medical indications. But the recently proposed restrictions on abortion are meant to prohibit the 95% of procedures that are done for nonmedical reasons.

In the last decade I have seen too many physicians who have been intimidated into silence by vocal spokespersons for abortion on demand. Now they demand our unconditional support as they suffer reverses in the ongoing battle in the courts and legislatures. The authors of this Clinical Opinion, as well as many others in our profession, are unwilling to give an inch in their support of abortion on demand. In spite of their intransigence, they should not be surprised to find that, as the tide turns, the right to privacy will not include the unrestricted right to abort a fetus.

Steve Calvin, MD
5806 Oakland Ave. S., Minneapolis, MN 55417

Webster should not affect prenatal diagnosis

To the Editors: We note with some concern remarks made by Chavkin and Rosenfield in their recent article (Chavkin W, Rosenfield A. A chill wind blows: Webster, obstetrics, and the health of women. Am J Obstet Gynecol 1990;163:450-2). They state, "Implicit in offering α-fetoprotein screening, chorionic villus sampling, amniocentesis for karyotyping, and other probes for specific fetal abnormalities is the option of termination of pregnancy. Without such an option, there is little rationale for carrying out these testing procedures." Although we have no argument with a careful reading of the first sentence, we do disagree with the second sentence. With this statement, Chavkin and Rosenfield are not considering the value of prenatal diagnostic information for the management of labor and delivery, anticipation of the need for (or futility of) neonatal care, or the increasing possibilities for in utero treatment of the abnormal fetus. Nor are they considering that in cases in which fetal death is a possibility, current prenatal diagnostic techniques may be the last op-

portunity to make accurate diagnoses so that the parents can make informed decisions about future reproduction.

Like Chavkin and Rosenfield, we are eager to offer women a full range of reproductive options, but we think that the issues should not be clouded by such ill-considered statements as the second sentence cited above. A considerable number of our patients do opt, for a variety of reasons, to continue their pregnancies with the knowledge that the fetus is not normal. We offer these women our full support of their decision, just as we offer our full support if they decide that termination is the best choice for them.

Chavkin and Rosenfield also express their concern that the Webster decision may lead to limitations of the obstetrician's ability to "counsel patients about the option or *advisability* of termination of pregnancy" [emphasis added]. We take issue with any obstetrician who advises a woman to have an abortion solely because her fetus is abnormal, and a number of our patients have reported that this is what has happened to them. Certainly a case can be made for advising termination of pregnancy when the pregnancy has an adverse effect on the mother's health. For fetal abnormality, abortion should be presented as one option in the context of all available options.

Mason Barr, Jr., MD, Robert H. Hayashi, MD, H. Frank Andersen, MD, Gabriella C. Pridjian, MD, and Clark E. Nugent, MD

Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Michigan, Ann Arbor, MI 48109

Reply

To the Editors: Dr. Calvin has written a generally critical letter concerning our recent article on abortion and the Supreme Court's Webster decision. He chastises us for raising the "specter of thousands of dead women." Perhaps he is unaware that illegal abortion is estimated by the World Health Organization to account for between 20% and 40% of the approximately 500,000 pregnancy-related deaths that are estimated to occur each year in the developing world. Furthermore, he must not know the data from the United States, which demonstrated a dramatic decline in death from illegal abortion after the legalization of abortion in the early 1970s.

He also misses our point when he states that medically indicated abortions are rare. It is the *purpose* of prenatal diagnosis that is threatened by these restrictions on abortion, even if relatively few procedures result in a positive diagnosis. Screening tests that are applied to populations will, of course, detect far fewer cases than people who are screened. Such screening tests are useful because of the possibility of subsequent action if they prove positive, which in this case would include the option of termination of the pregnancy.

Dr. Barr and colleagues express concern that we presume abortion to be the sole course of action after an abnormal prenatal diagnosis. This is not the case; we concur with them that such a diagnosis can be useful to medical management and future planning and that such decisions must be the patient's. Furthermore, we agree that, in counseling patients, the appropriate approach is to discuss options and not to suggest what is advisable. However, we also believe that the efficacy and availability of therapeutic possibilities for fetal abnormalities remains limited at the present time. We maintain therefore that the option of abortion is essential for prenatal diagnostic efforts to be a meaningful component of standard prenatal care. Moreover, future explorations in prenatal diagnosis and other aspects of reproductive medicine will be severely constricted if the Webster prohibition against termination after fertilization is deemed enforceable.

Our basic point here, however, is a larger one, namely, that the kinds of governmental restrictions that are exemplified by the Webster decision are not medically sound nor are they appropriate for governmental control. They place restrictions on the long-standing confidential relationship between a physician and his or her patient and interfere with the scientific basis on which the practice of medicine is based.

Furthermore, the impact of the Webster decision will be to aggravate disparities in health status and access to care as it is related to income. By effectively imposing delay on poor women who seek abortions, the State of Missouri imposes health risks on the very group that is already at highest risk.

Our article did not address abortion as an abstract or moral issue, a matter on which we and Dr. Calvin clearly disagree. Rather, our effort was to attempt to delineate for obstetrician-gynecologists some of the ways in which Webster-style restrictions on abortion will undermine the autonomy and integrity of our profession.

Wendy Chavkin, MD, MPH, and Allan Rosenfield, MD

Columbia School of Public Health and the Department of Obstetrics and Gynecology, Columbia Presbyterian Medical Center, 600 West 168th St., New York, NY 10032

Correction

In the article by Manabe and Yoshida, "Morphologic study of changes of collagenous tissue in the amnion and cervix during prostaglandin-induced abortion and delivery" (AM J OBSTET GYNECOL 1990;163:52-6), Fig. 1 on page 53 was printed upside down. As a result, the legend was incorrect; the figure information was correct from bottom to top rather than from top to bottom.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

The IXth International Congress of Endocrinology, Acropolis, Convention Center, Nice, France, August 30 to September 5, 1992. For information contact: IXth International Congress of Endocrinology, SOCFI, 14, Rue Mandar, 75002 Paris, France. Tel.: 33 (1) 42 33 89 94. Fax: 33 (1) 40 26 04 44.

Thirteenth Course in Colposcopy and Cervico-Vaginal Histopathology (in English): Demonstration of the Use of Diathermy Loop, Paris, September 30–October 4, 1991. For information contact: Dr. Cartier, 20 Rue des Cordelières, 75013 Paris, France. Tel.: 33.1.43.36.04.46.

Postgraduate Course in Gynecologic and Obstetric Pathology with Clinical Correlation, April 8-12, 1991, Parker House Hotel, Boston, Massachusetts. Fee for course is $650.00 (residents and fellows, $450.00). Address for further information: Department of Continuing Education, Harvard Medical School, 25 Shattuck St., Boston, MA 02115.

Infertility Update VI, March 9, 1991, Scripps Clinic and Research Foundation, La Jolla, California. For more information contact: Susan J. Buntjer, Conference Coordinator, Department of Academic Affairs, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037. Tel.: (619) 554-8556.

3rd Annual Review Course in Obstetrics and Gynaecology, April 30–May 3, 1991, Delta Chelsea Inn, Toronto, Ontario, Canada. AMA category I study credits. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.

International Symposium on Advances in Contraception and Contragestion, Lausanne, Switzerland, October 2-4, 1991. For further information contact: Dr. S. Kaul, MEDAVICE, P.O. Box 82, CH-4105 Biel-Benken, Switzerland. Fax: (061) 73 16 12; (061) 721 16 12 after April 22, 1991.

Office Gynecology, April 15-19, 1991, The Hyatt of Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.

Chicago Area Schools of Medicine Obstetrics and Gynecology Review Course, June 3-8, Westin Hotel, Chicago, Illinois. Credit: 44 hours Category I, 44 Cognates, Formal Learning, ACOG. For information contact: The University of Chicago, Center for Continuing Medical Education, 5841 S. Maryland Ave., Box 139, Chicago, IL 60637. Tel.: (312) 702-1056.

The 8th International Congress of Human Genetics, sponsored by the American Society of Human Genetics, will be held October 6-11, 1991, at the Washington Convention Center in Washington, D.C. The deadline for receipt of abstracts is April 1, 1991. For abstract and registration forms or additional information contact: M. Ryan, Meetings Manager, ICHG, 9650 Rockville Pike, Bethesda, MD 20814. Tel.: (301) 571-1825; Fax (301) 530-7079.

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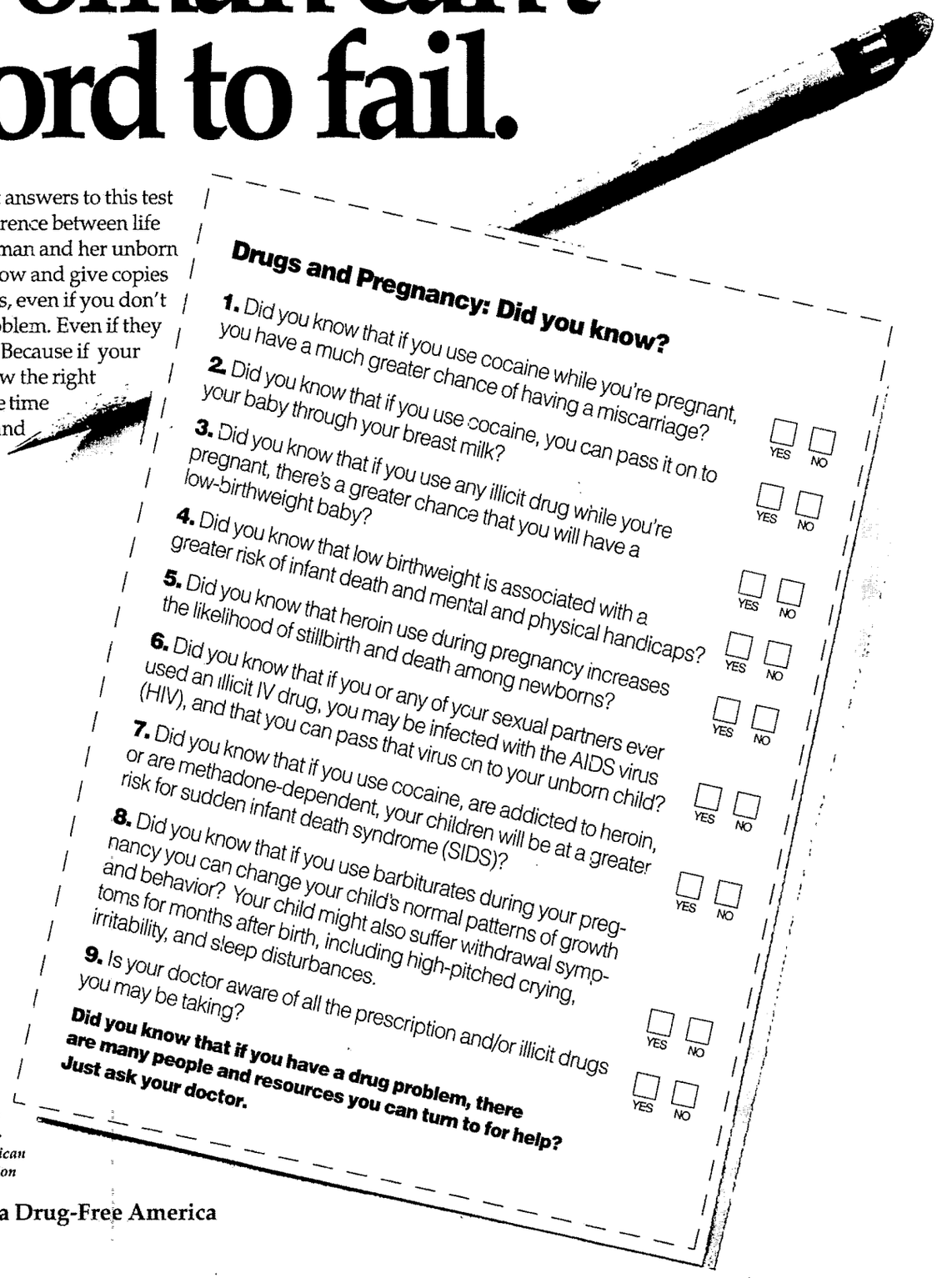
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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

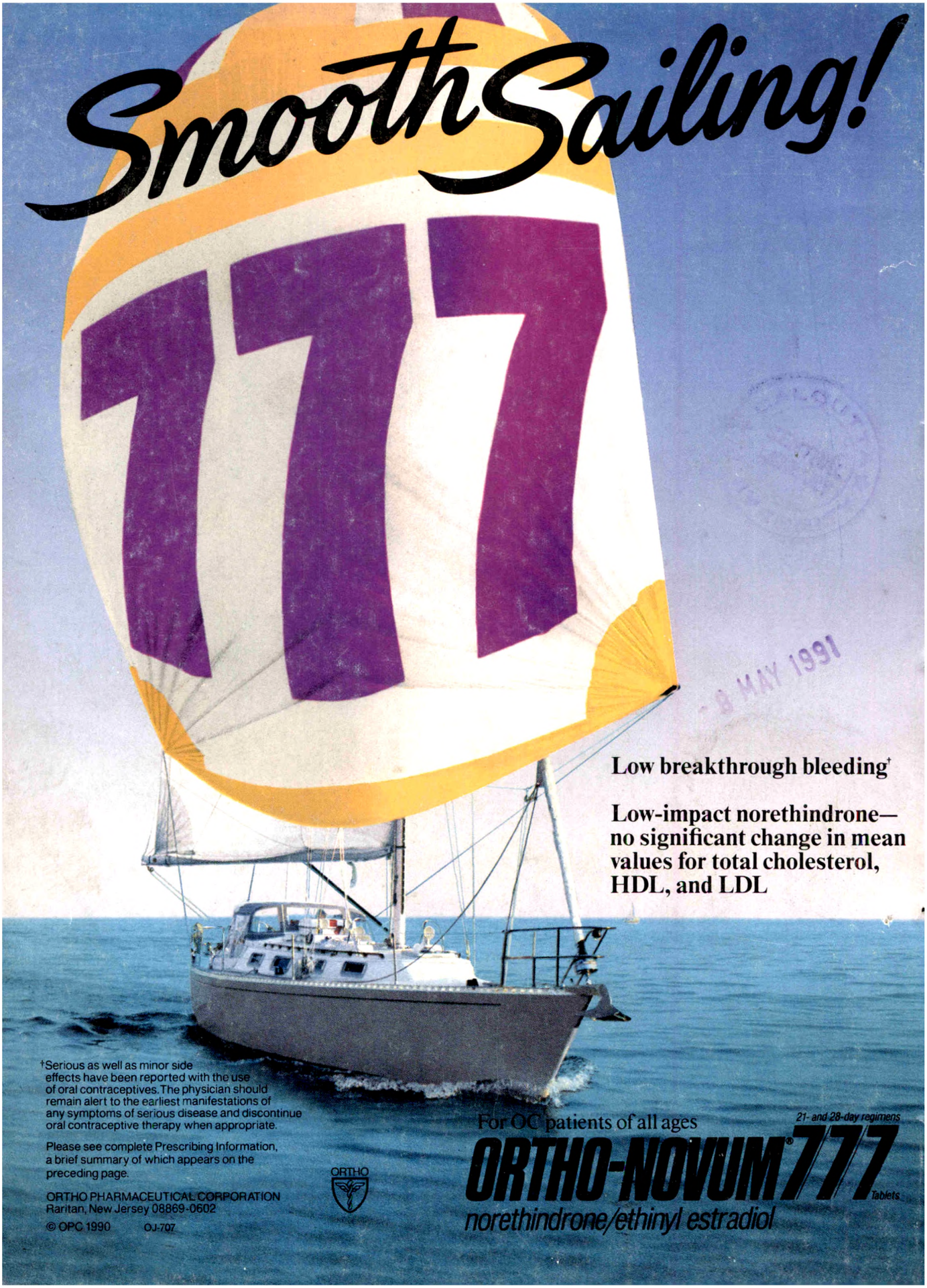
Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Revised 9/89

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Raritan, NJ 08869-0602

ORTHO


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777
Low breakthrough bleeding†
Low-impact norethindrone—no significant change in mean values for total cholesterol, HDL, and LDL
†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.
Please see complete Prescribing Information, a brief summary of which appears on the preceding page.
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ORTHO
For OC patients of all ages
21- and 28-day regimens
ORTHO-NOVUM® 777 Tablets
norethindrone/ethinyl estradiol

March 1991
volume 164, *number* 3

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(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL®

Uniquely phased for all phases of a woman's reproductive life

ORAL CONTRACEPTIVE AGENTS: BRIEF SUMMARY

DESCRIPTION

TRI-NORINYL 21-DAY Tablets provide an oral contraceptive regimen of 7 blue tablets followed by 9 yellow-green tablets and 5 more blue tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg and each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg.

TRI-NORINYL 28-DAY Tablets provide a continuous oral contraceptive regimen of 7 blue tablets, 9 yellow-green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg, each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert ingredients.

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions: Thrombophlebitis or thromboembolic disorders • A past history of deep vein thrombophlebitis or thromboembolic disorders • Cerebral vascular or coronary artery disease • Known or suspected carcinoma of the breast • Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia • Undiagnosed abnormal genital bleeding • Cholestatic jaundice of pregnancy or jaundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors • Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholesterolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estrogen. The effects of long-term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the *ratio* of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide not only a measure of the relative risk but a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

a. Myocardial infarction

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS,** section 9). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2- to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970's—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral contraceptive use to women who do not have the various risk factors listed in this labeling.

Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular disease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users.

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0.075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed earlier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The incidence of risk also was reported to increase with continued use and among older women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens.

Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out.

Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. LIPID DISORDERS

Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention.

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected by oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.

c. Other binding proteins may be elevated in serum.

d. Sex steroid binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged.

e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives.

9. CARCINOGENESIS

See **WARNINGS** section.

10. PREGNANCY

Pregnancy Category X. See **CONTRAINDICATIONS** and **WARNINGS** sections.

11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

INFORMATION FOR THE PATIENT

See **PATIENT LABELING.**

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **WARNINGS** section): Thrombophlebitis • Arterial thromboembolism • Pulmonary embolism • Myocardial infarction • Cerebral hemorrhage • Cerebral thrombosis • Hypertension • Gallbladder disease • Hepatic adenomas, carcinomas or benign liver tumors

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis • Retinal thrombosis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea • Vomiting • Gastrointestinal symptoms (such as abdominal cramps and bloating) • Breakthrough bleeding • Spotting • Change in menstrual flow • Amenorrhea • Temporary infertility after discontinuation of treatment • Edema • Melasma which may persist • Breast changes: tenderness, enlargement, secretion • Change in weight (increase or decrease) • Change in cervical erosion and secretion • Diminution in lactation when given immediately postpartum • Cholestatic jaundice • Migraine • Rash (allergic) • Mental depression • Reduced tolerance to carbohydrates • Vaginal candidiasis • Change in corneal curvature (steepening) • Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome • Cataracts • Changes in appetite • Cystitis-like syndrome • Headache • Nervousness • Dizziness • Hirsutism • Loss of scalp hair • Erythema multiforme • Erythema nodosum • Hemorrhagic eruption • Vaginitis • Porphyria • Impaired renal function • Hemolytic uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral contraceptive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: • Increased menstrual cycle regularity • Decreased blood loss and decreased incidence of iron deficiency anemia • Decreased incidence of dysmenorrhea

Effects related to inhibition of ovulation: • Decreased incidence of functional ovarian cysts • Decreased incidence of ectopic pregnancies

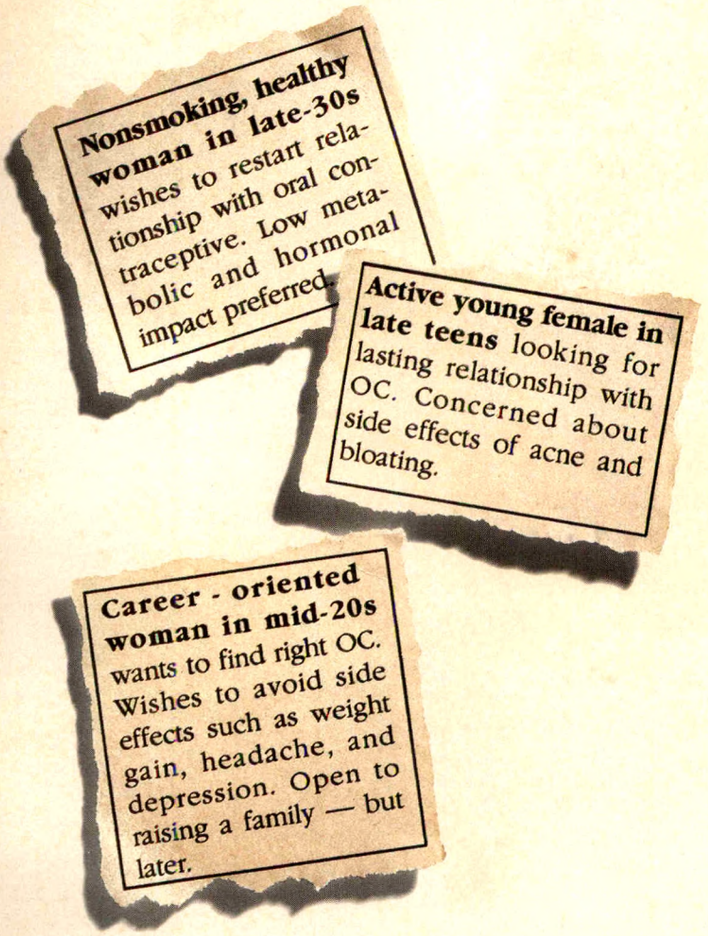
Effects from long-term use: • Decreased incidence of fibroadenomas and fibrocystic disease of the breast • Decreased incidence of acute pelvic inflammatory disease • Decreased incidence of endometrial cancer • Decreased incidence of ovarian cancer

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814-H2-170-90

Rev. May 1990

SYNTEX

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A personalized approach to oral contraceptives.

Because Tri-Norinyl is uniquely phased for all phases of a woman's reproductive life, it's ideal for a wide range of patient types:

Older, nonsmoking patients restarting contraception: The only multiphasic with norethindrone that *lowers* progestin in late cycle to minimize overall hormonal and metabolic impact.

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Younger patients just starting contraception: Low-impact progestin lowers risk of acne and bloating; mid-cycle increase enhances support when breakthrough bleeding is most likely to occur.

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Uniquely phased for all phases of a woman's reproductive life

Committed to the OB/GYN and women's healthcare

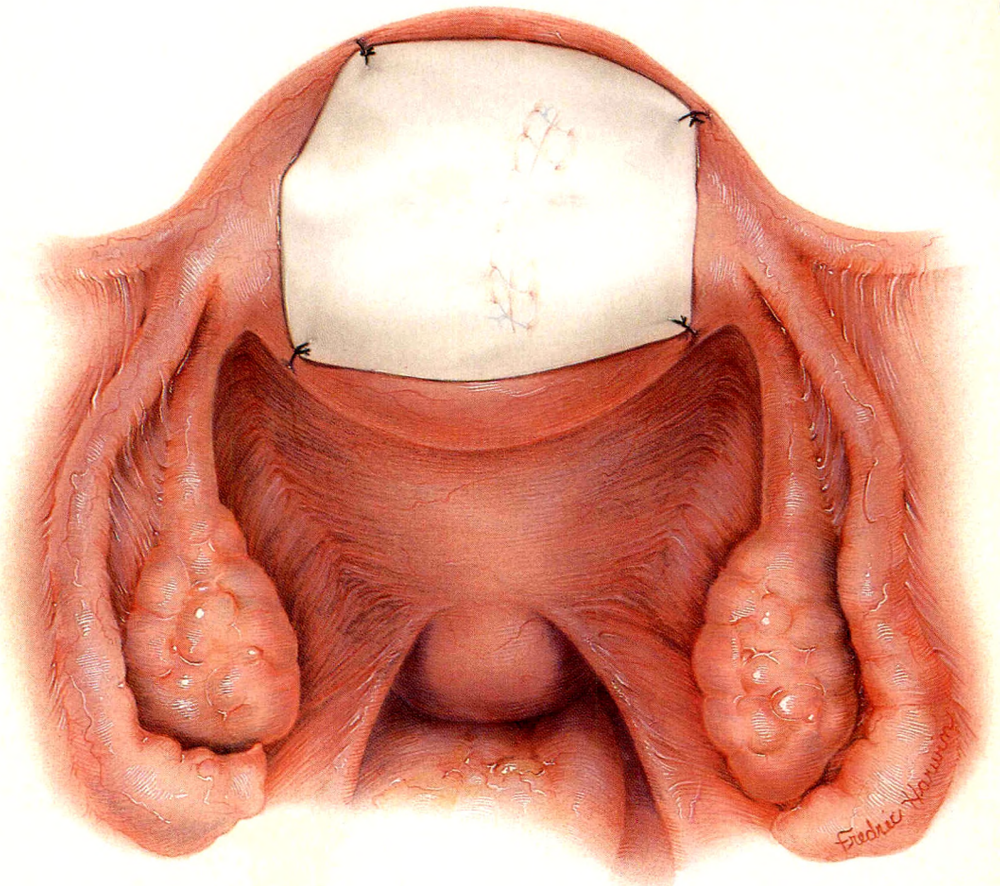
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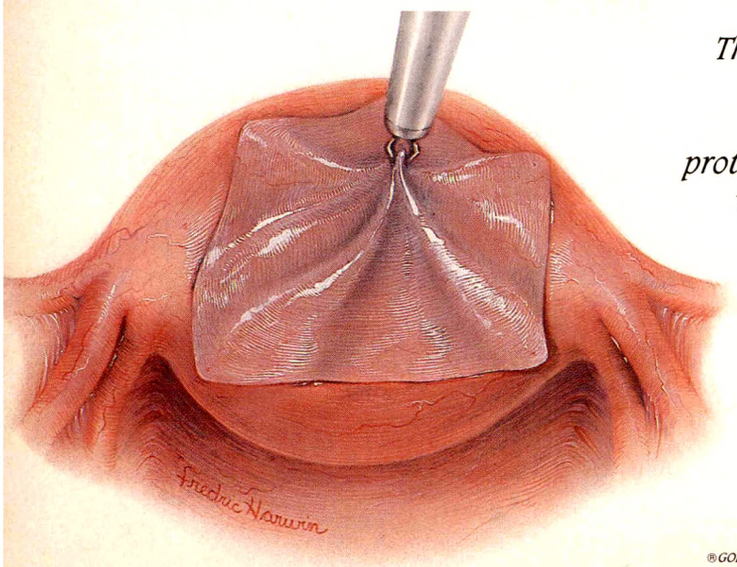
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Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see next page for brief summary of prescribing information. ©1990 Syntex Laboratories, Inc. 814-H2-201-90

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Invariant Performance
Minimizing



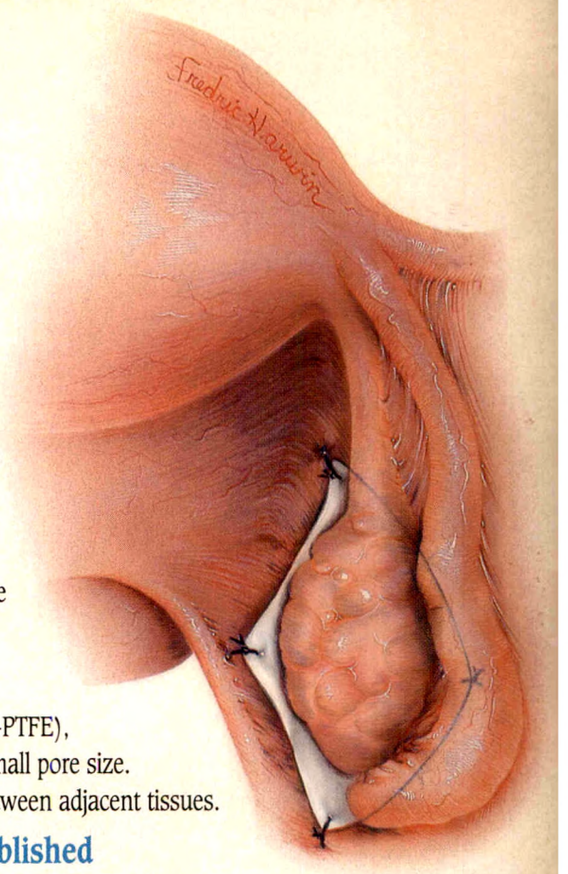


The GORE-TEX Surgical Membrane –
Configured for
Peritoneal Reconstruction
protects denuded areas from re-adhesion
while healing progresses beneath
the material.

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The Choice in Peritoneal Reconstruction



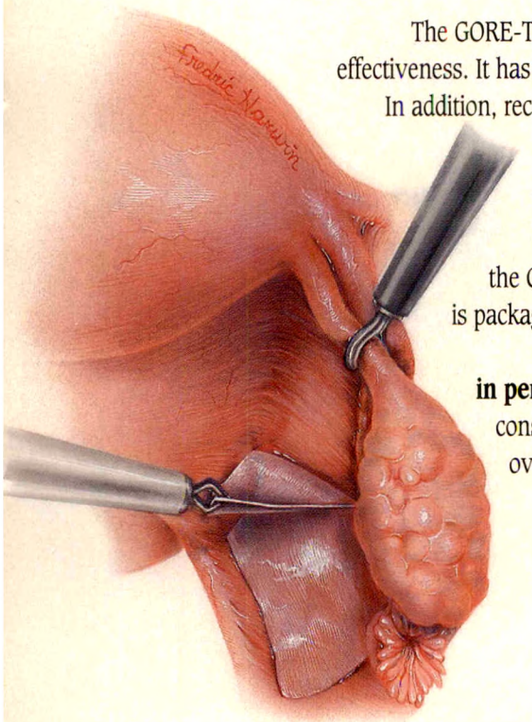
Successful peritoneal reconstruction requires dependable measures to minimize adhesion reformation following adhesiolysis. The invariant performance of the GORE-TEX Surgical Membrane aids in producing consistent results.

Performance by Design

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The GORE-TEX Surgical Membrane has a long record of safety and effectiveness. It has demonstrated reliability among cardiac surgeons for more than 14 years. In addition, recent use in a multi-center clinical study has substantiated safety and shown invariant performance in peritoneal repair.



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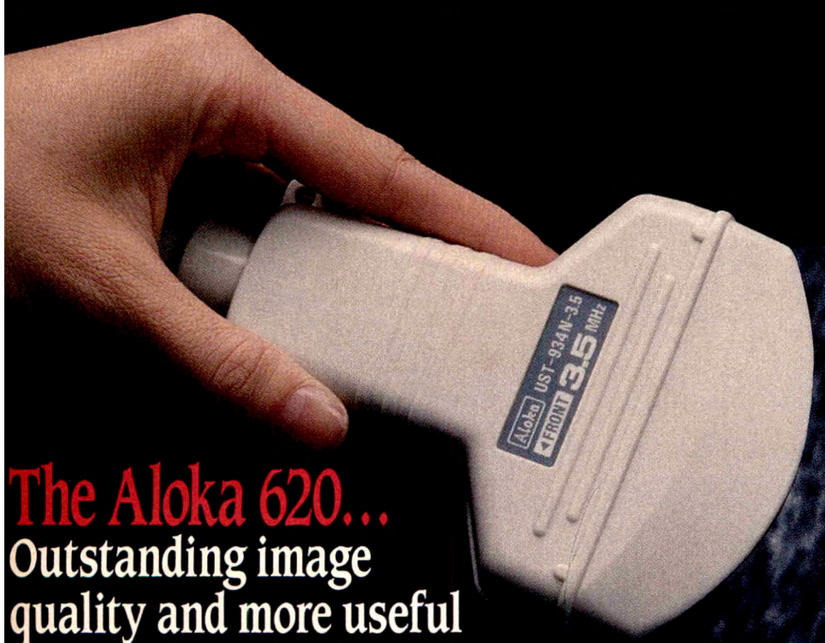
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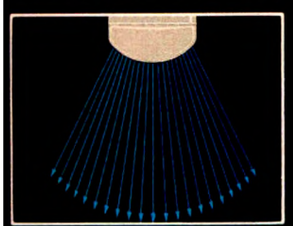
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American Journal of Obstetrics and Gynecology

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Seattle, Washington, Bethesda, Maryland, Research Triangle Park, North Carolina, San Antonio, Texas, New Orleans, Louisiana, Oklahoma City, Oklahoma, and New York, New York

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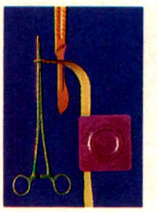
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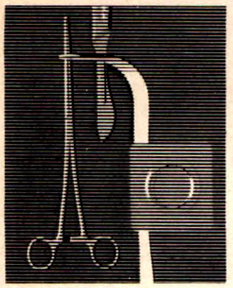
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In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

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In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary tract infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).
Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli.*
Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli.*
Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).
Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).
Bone and joint infections caused by *Staphylococcus aureus.**
*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.

PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.
INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.
DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.
CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be

developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.
USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.
USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.
PEDIATRIC USE: Safety and effectiveness in children have not been established.
ADVERSE REACTIONS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).
Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).
Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).
Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).
Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.
DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.
IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:} \quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.
HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.

1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

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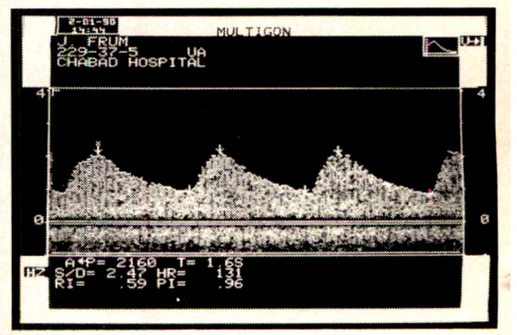
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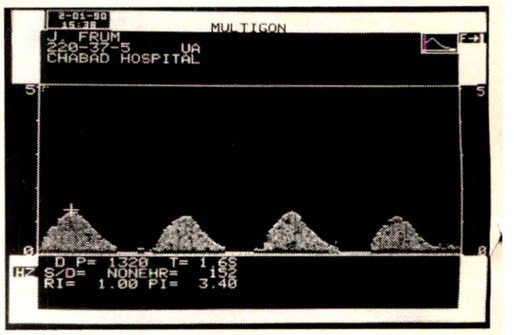


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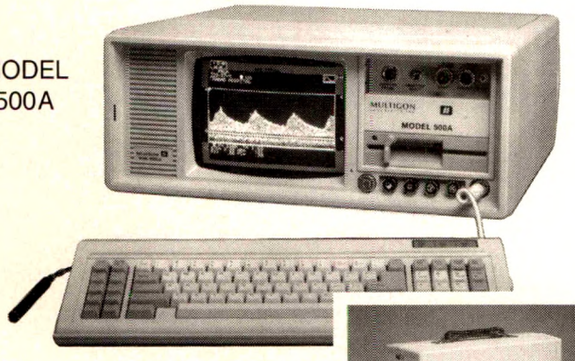


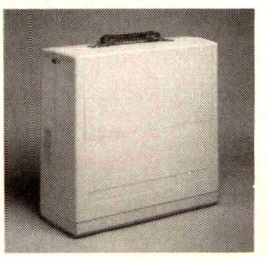
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Berkeley, San Francisco, and San Diego, California

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Dean S. Cunningham, MD, PhD, David L. Fulgham, BS, David L. Rayl, MD, Keith A. Hansen, MD, and Nancy J. Alexander, PhD
Portsmouth and Norfolk, Virginia

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Catherine Derom, PhD, Robert Vlietinck, MD, Robert Derom, MD, Charles Boklage, PhD, Michel Thiery, MD, and Herman Van den Berghe, MD
Leuven and Ghent, Belgium, and Greenville, North Carolina

The analysis of deoxyribonucleic acid extracted from the placenta allows for accurate genotyping of macerated stillborn fetuses.

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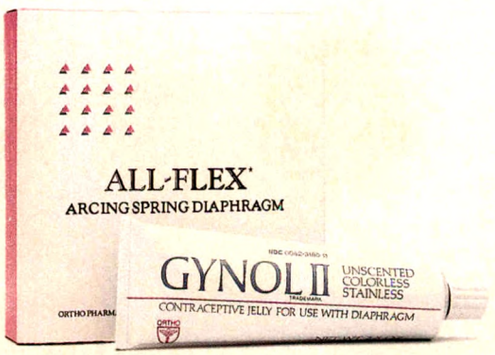
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San Francisco and Oakland, California

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Brian Kirshon, MD, Bernard Rosenfeld, MD, Giancarlo Mari, MD, and Michael Belfort, MD
Houston, Texas

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Alistair B. Roberts, MD, Israel Goldstein, MD, Roberto Romero, MD, and John C. Hobbins, MD
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Ann Arbor, Michigan

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Rome and Ancona, Italy

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San Francisco, California, and Seattle, Washington

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New Haven, Connecticut

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Salt Lake City, Utah, Houston and San Antonio, Texas, Seattle, Washington, and Los Angeles, California

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Kawagoe and Tokyo, Japan

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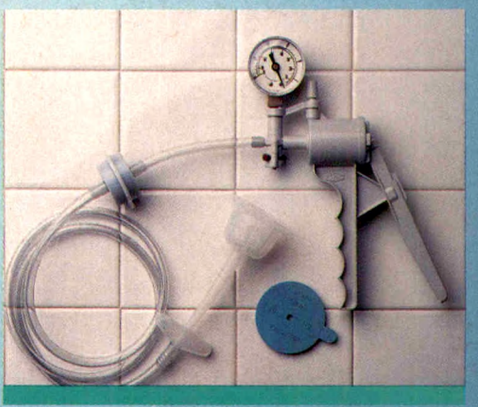
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Dallas, Texas

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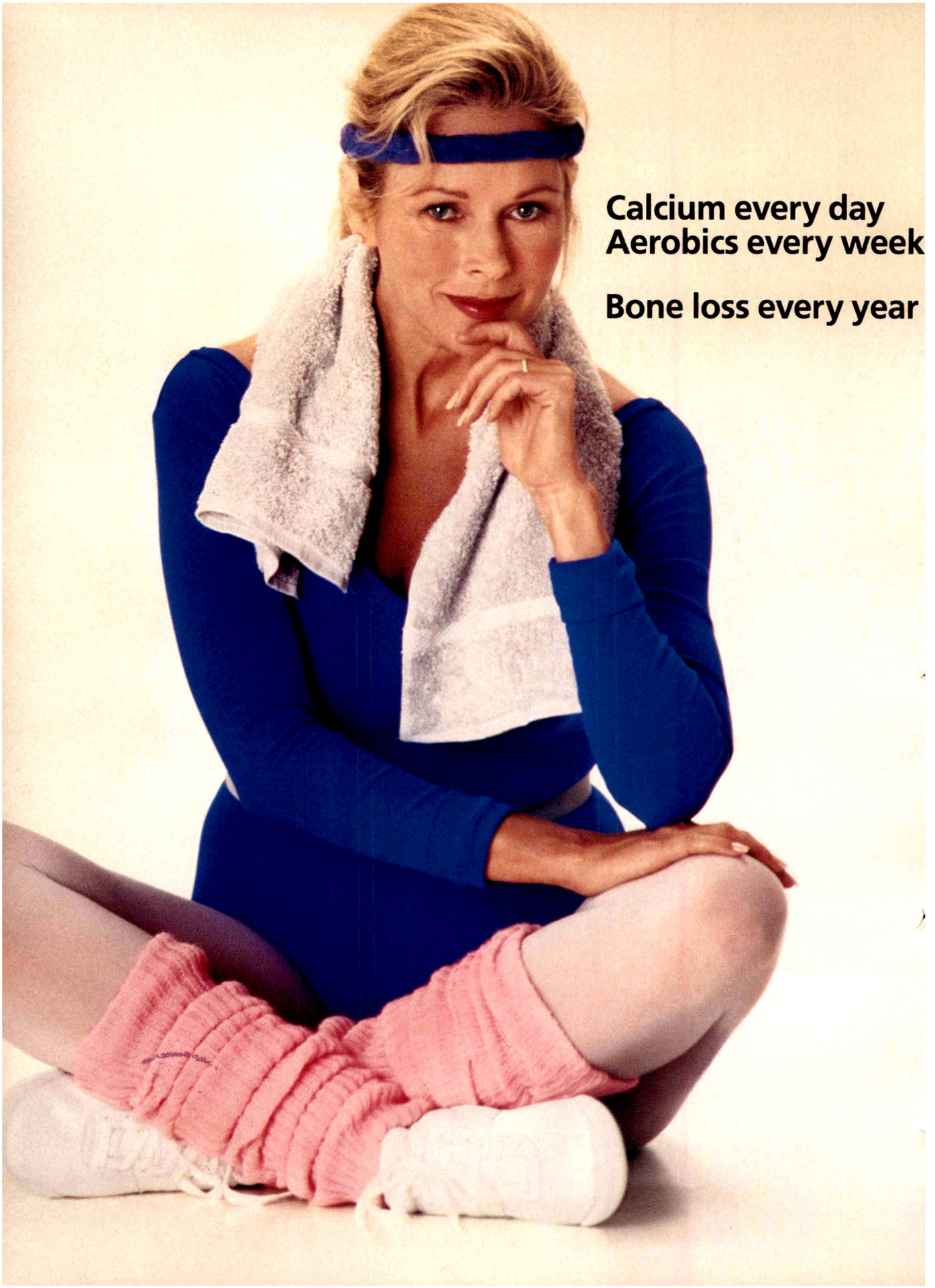
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See important information on adjacent page.

For moderate-to-severe vasomotor symptoms and for osteoporosis

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Vaginal Cream

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BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA. Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY. Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.

There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risk, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy; changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.

Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

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Tel.: (614) 293-8798

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INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1988;108:258-65. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL.

Manuscript submission. Manuscripts should be submitted to one of the two Editors in Chief as follows:

1. **Dr. Zuspan**—manuscripts from the eastern United States and from England, Israel, and Italy, manuscripts written for Clinical Opinion and Current Development, Letters to the Editors, and all manuscripts presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation. *Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. Donald R. Coustan, Department of Obstetrics and Gynecology, Women and Infants Hospital of Rhode Island, 101 Dudley St., Providence, RI 02905.*
2. **Dr. Quilligan**—manuscripts from states west of Pennsylvania, West Virginia, Kentucky, Tennessee, and Mississippi; manuscripts from Hawaii, Alaska, Canada, and abroad (except England, Israel, and Italy). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. *Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.*

Author's designation of reviewers. When authors submit their manuscripts, they should provide the names and addresses of at least three reviewers for consideration by the Editors.

Copyright statement. The specified copyright statement that follows the Information for Authors in each issue of the JOURNAL must be completed, signed by all authors, and submitted with the manuscript. (The Checklist and Copyright Statement may be torn from the JOURNAL or photocopied for submission.) If not completed in full, it will be returned to the author for completion.

Checklist. The checklist that appears with the copyright statement in each issue of the JOURNAL must be fully completed by the author(s) and sent with each submitted manuscript. A more complete description of each item that must be checked is provided under the appropriate heading in the Information for Authors, which is published in each issue of the JOURNAL. *If the checklist is not completed in full, the deficiencies will be noted, and the form, as well as the manuscript when necessary, will be returned to the author for completion.*

Previous publication. If a report by the same author(s) has been previously published in any medium that deals in any respect whatever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. The author(s) should inform the Editor of the circumstances, similarities, and differences of the reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

Human and animal experimentation. It is assumed by the Editors that manuscripts emanating from a particular institution are submitted with the approval of the requisite authority. Human experimentation that requires local institutional approval must have this approval *before the ex-*

periment is started and approval must be so indicated in the Methods section of the submitted manuscript. Reports of experiments on animals must *state in the Methods section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed. The species of nonhuman animals used in an investigation must be named in the title, abstract, and key words of the manuscript.

Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.

Conflict of interest. Authors are expected to inform the Editor, in a letter accompanying the submitted manuscript, of any commercial association that might pose a conflict of interest, such as ownership, stock holdings, equity interests and consultant activities, or patent-licensing situations. Such information is confidential, is not given to the consultants, and does not play a part in the decision of the quality or timeliness of the manuscript. If the manuscript is accepted, the author and the Editor will determine how best to release the information. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest; only where there is a possible conflict of interest is the author(s) expected to inform the Editor.

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General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of glossy prints of illustrations are required.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, figure legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable, but honors degrees are not permitted); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, business and home telephone numbers, and fax number of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, business and home telephone numbers, and fax number of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single-spaced sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page and key words/phrases. On manuscript page 3, type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare

Estimating length of manuscripts

With the exception of Current Development articles, the length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number (25) is allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (Ann Intern Med 1988;108:258-65). Journal titles should conform to abbreviations used in *Cumulated Index Medicus*.

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

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BOOKS: Ledger WJ. Dystocia and prolonged labor. In: Wilson JR, Carrington ER, eds. Obstetrics and gynecology. St. Louis: CV Mosby, 1987 vol 8:474-93.

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Figures. The term *figure* includes all types of illustrations such as graphs, diagrams, photographs, flow charts, and line drawings. A reasonable number of figures will be reproduced without charge, but special arrangements must be made with the Editors for color figures at an additional charge to the author. The quoted charge is per page of color, and as many as eight color figures can be included on one page.

Figures must be cited consecutively in the text in Arabic numerals and identified with a gummed label on the back of the figure with author(s) name(s), title of article, figure number, and top marked clearly. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted. Do not use paper clips or mar the surface of figures in any way. Figures will be returned only on request by the author.

For *black-and-white figures,* submit three sets of 3 × 4 inch (minimum) to 5 × 7 inch (maximum) unmounted, glossy prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy prints with good black-and-white contrast.

For *color figures* submit original transparencies and two sets of unmounted prints on glossy (smooth surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The top, first author's last name, and figure number must be indicated on the front of each transparency and the back of each print.

Computer-generated figures must be legible and clearly printed in jet black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend should not appear on the figure. On the back of each figure the name of the first author and the figure number should be given and the top indicated. Three original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color figures* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color figures.The preparation and submitting of color prints should fol-

low the preceding guidelines for black-and-white computer-generated illustrations.

Legends to figures. Legends for all figures must be typed double-spaced on paper separate from the text of the manuscript, and these pages must be numbered in sequence after the references. Titles should be included in the legend, not on the print. Original magnifications should be provided. If a figure has been taken from copyrighted material, the legend must give full credit to the original source.

Tables. Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Titles should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. If a table or any part thereof has been taken from copyrighted material, a footnote to the table must give full credit to the original source. Special arrangements must be made with the Editors for elaborate tables because of space limitations.

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Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

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Two types of letters will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating "Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60 U.S., and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

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These items appear in each monthly issue of the JOURNAL. A photocopy may be used. An exposition of the requirements given in the checklist is in the Information for Authors, which is published in each issue of the JOURNAL.

CHECKLIST

General

—The original and two copies of the manuscript are submitted.
—The appropriate signed completed checklist and assignment of copyright signed by **all** authors accompany the manuscript.
—Three sets of unmounted glossy prints for regular figures are submitted, properly numbered and labeled on the back of each print.
—Three sets of original computer-generated figures printed on heavy coated paper with either a glossy or dull finish, unmounted, each one on a page, and properly labeled on the back are submitted. None are dot matrix or photographic prints.
—Human experimentation has been approved by the local institution as stated in the Methods section.
—Guidelines for the care and use of animals approved by the institution have been followed as indicated in the Methods section.
—The covering letter with essential information is with the manuscript.
—All elements of the manuscript are typed double-spaced on bond paper with 1-inch margins at top, bottom, and sides.
—All pages are numbered in the following order: title page, condensation, abstract, body of the text, acknowledgments only of persons who have made substantive contributions to the study, references, legends, and tables.
—Signed, written permission from both the copyright holder and the original author for the use of tables, figures, or quotations previously published and their complete references are enclosed with the manuscript.
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—On a separate sheet of paper, page 2 of the manuscript, a single sentence limited to 25 words delineating the essential point(s) is typed double-spaced.

Abstract

—The abstract is typed double-spaced with required margins (on page 3) headed by the title and the author(s) name, not exceeding 150 words. For case reports and brief communications the limit of 50 words is not exceeded.
—Three to five key words or short phrases are placed beneath the abstract on page 3.

Tables

—Each table headed by a title and numbered in Roman numerals is typed double-spaced on a separate page.

—Tables are cited in sequence in the text.

Figures

—Each is numbered with an Arabic numeral and cited in numerical sequence in the text.

—Original transparencies of **color photographs** appropriately numbered and identified are submitted along with two sets of unmounted prints on glossy paper, numbered and identified on the back.

References

—Are typed double-spaced.

—Are numbered consecutively in the order they are cited in the text.

—The format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" is used. Examples shown in Information for Authors were followed.

—Personal communications, and unpublished work are not used as numbered references but are mentioned in the text with the written approval of the person being quoted. The signed approval is enclosed.

AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

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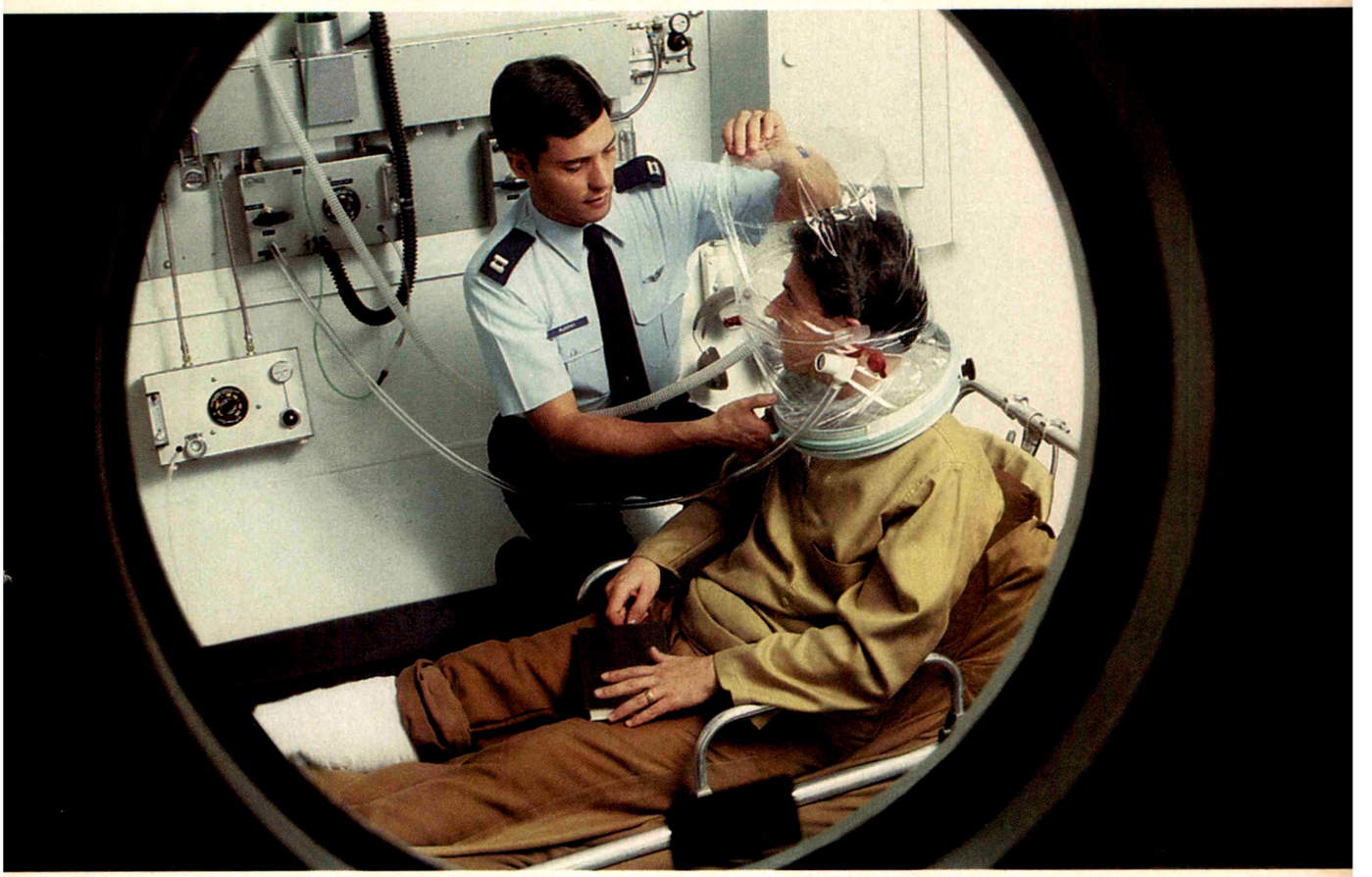
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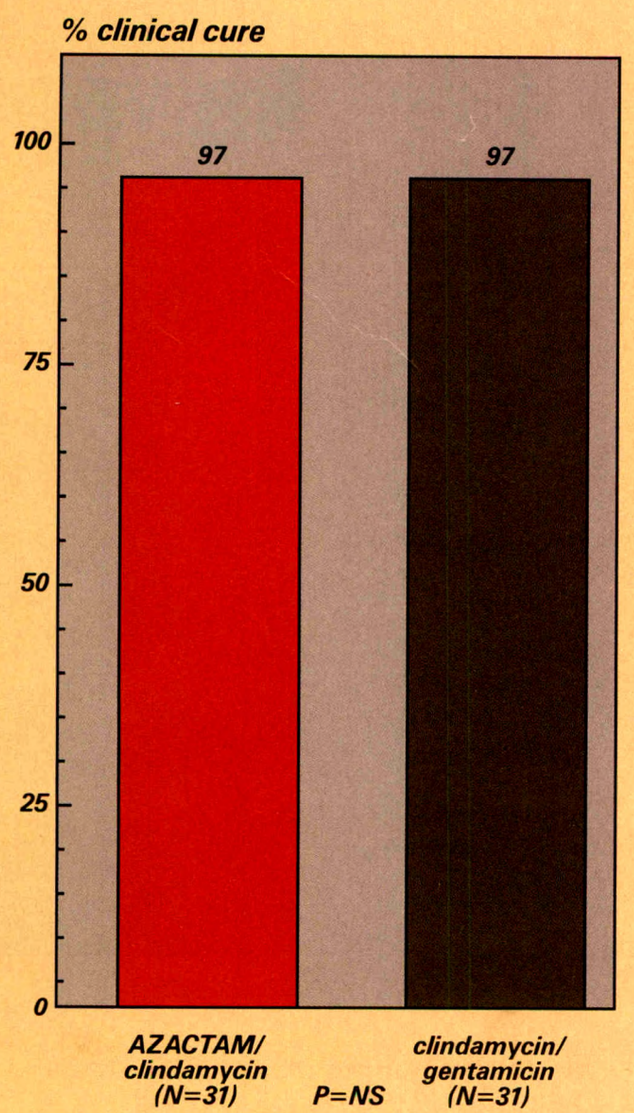
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AIM HIGH.

AIR FORCE

IN POSTPARTUM ENDOMETRITIS*



A randomized, comparative trial of AZACTAM versus gentamicin, each with clindamycin, for the treatment of endometritis after delivery.[1]

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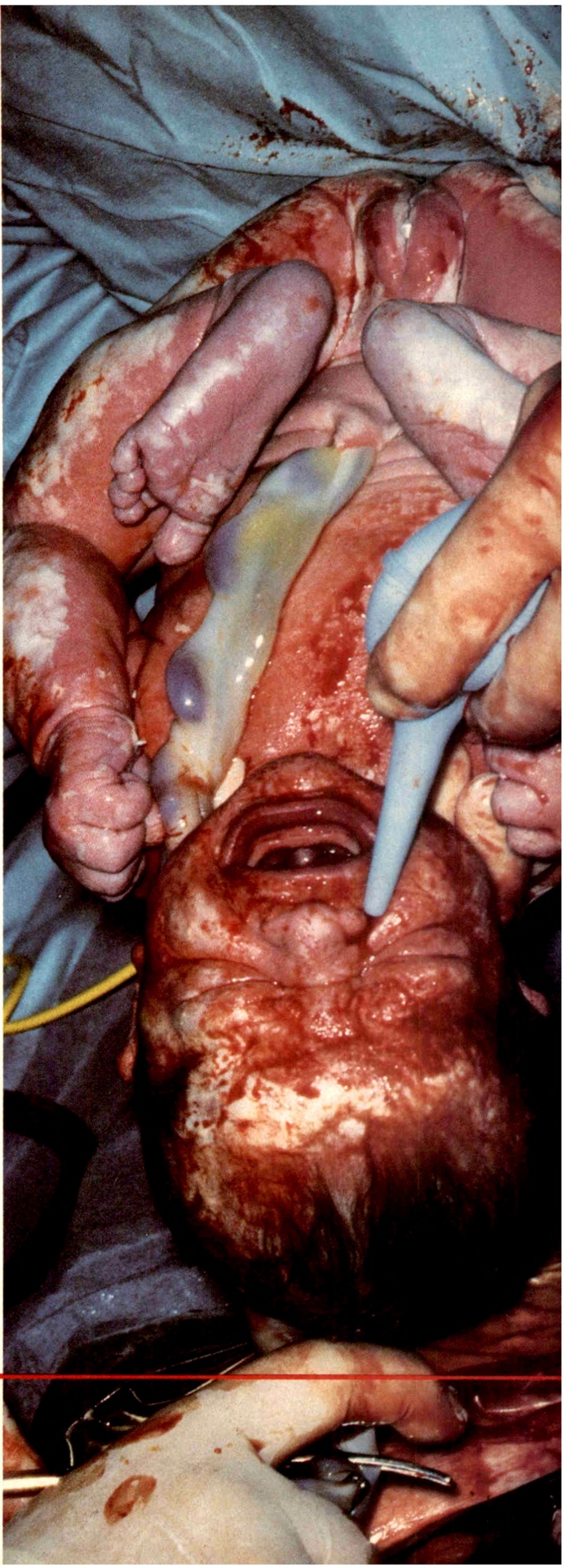
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- *Pregnancy Category B.*

There are no adequate and well-controlled studies in pregnant women. AZACTAM should be used in pregnant women only if clearly needed.



Azactam®
aztreonam

A logical alternative to aminoglycosides.

Please see adjacent page for brief summary of prescribing information.

Azactam®
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AZACTAM® FOR INJECTION
Aztreonam For Injection

DESCRIPTION–AZACTAM (aztreonam, Squibb) is the first member of a new class of antibiotics classified as monobactams. AZACTAM is a totally synthetic bactericidal antibiotic with activity against a wide spectrum of gram-negative aerobic pathogens. The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics.

AZACTAM For Injection is a sterile, nonpyrogenic, sodium-free, white to yellowish-white lyophilized cake, containing approximately 780 mg arginine per gram of aztreonam for intramuscular or intravenous use following constitution. Aqueous solutions of the product have a pH in the range of 4.5-7.5.

INDICATIONS AND USAGE–Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: **Urinary Tract Infections** (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca*, Citrobacter* species* and *Serratia marcescens**. **Lower Respiratory Tract Infections,** including pneumonia and bronchitis caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens**. **Septicemia** caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*, Serratia marcescens** and *Enterobacter* species. **Skin and Skin-Structure Infections,** including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Citrobacter* species*. **Intra-abdominal Infections,** including peritonitis caused by *Escherichia coli, Klebsiella* species, including *K. pneumoniae, Enterobacter* species including *E. cloacae*, Pseudomonas aeruginosa, Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**. **Gynecologic Infections,** including endometritis and pelvic cellulitis caused by *Escherichia coli, Klebsiella pneumoniae*, Enterobacter* species* including *E. cloacae**, and *Proteus mirabilis**.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy–Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organisms(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an antianaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS–Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS–Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin). Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

PRECAUTIONS–General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility–Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy–Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers–Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use–Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS–Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity; *Hypersensitivity*–anaphylaxis. *Hematologic*–pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. *Gastrointestinal*–abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). *Dermatologic*–purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. *Cardiovascular*–hypotension, transient ECG changes (ventricular bigeminy and PVC). *Respiratory*–one patient experienced flushing, chest pain, and dyspnea. *Hepatobiliary*–hepatitis, jaundice. *Nervous System*–seizure, confusion, vertigo, paresthesia, insomnia, dizziness. *Musculoskeletal*–muscular aches. *Special Senses*–tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. *Other*–vaginal candidiasis, vaginitis, breast tenderness. *Body as a Whole*–weakness, headache, fever, malaise.

Adverse Laboratory Changes–Those reported without regard to drug relationship during clinical trials were: *Hepatic*–elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*–increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Renal*–increases in serum creatinine.

OVERDOSAGE–If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION–Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg,** or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231C)

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

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SQUIBB

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EDITORIAL

Summary of analysis of published articles

September 1, 1989, through August 31, 1990

Each year a retrospective analysis of the articles published in the JOURNAL is presented to the readers and also to the Editorial Board and the Advisory Committee on Policy. Over the past twenty-one years we have compiled data on the classification, category, and type of articles accepted and published. A comparison of the data for the last two years is presented in Table I. A comparison of publication time in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY of regular articles (excluding society articles) for 1985-1986 through 1989-1990 is shown graphically in Fig. 1, on page 710.* The pie charts on page 710 show the articles and page utilization for 1989-1990 by classification (Fig. 2A) and category (Fig. 2B).

*Publication time of regular articles from time of acceptance to publication: 1985-1986, 3.7 months; 1986-1987, 4.9 months; 1987-1988, 5.2 months; 1988-1989, 5.6 months; and 1989-1990, 5.1 months.

Table I. Comparison of data on classification, category, and type of articles published in 1988-1989 and 1989-1990

	1988-1989			*1989-1990*		
	No. of articles	*Pages*	*Average length per article*	*No. of articles*	*Pages*	*Average length per article*
Classification of articles:						
Clinical	519	2309		506	2407	
Basic Science	110	635		130	731	
Total	**629**	**2944**		**636**	**3138**	
Category of articles:						
Obstetrics	237	1086		253	1240	
Gynecology	156	764		151	770	
Fetus, Placenta, Newborn	225	1045		224	1091	
Education	11	49		8	37	
Total	**629**	**2944**		**636**	**3138**	
Composition of the JOURNAL based on the type of article:						
Regular	329	1609	4.9	331	1757	5.3
Society	185	1043	5.6	175	998	5.7
Brief Communication/Case Report	93	185	2.0	99	199	2.0
Current Investigation	1	4	4.0	2	15	7.5
Current Development	1	6	6.0	7	71	10.1
Clinical Opinion	20	97	4.9	22	98	4.5
Total	**629**	**2944**		**636**	**3138**	

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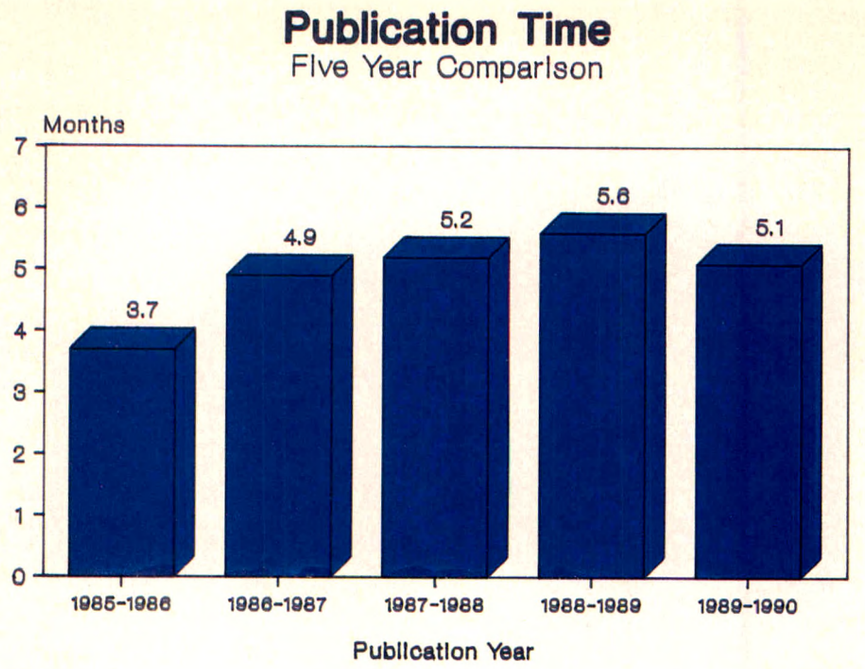


Fig. 1. Five-year comparison of publication time of regular articles (excluding society articles) in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY.

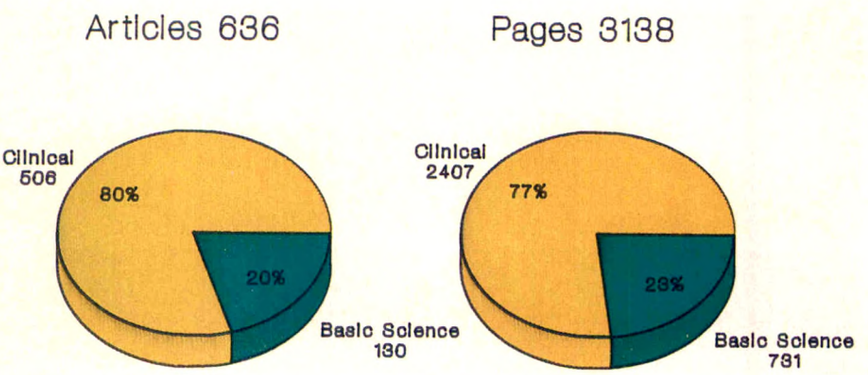


Fig. 2A. Articles and page utilization for 1989-1990 by classification.

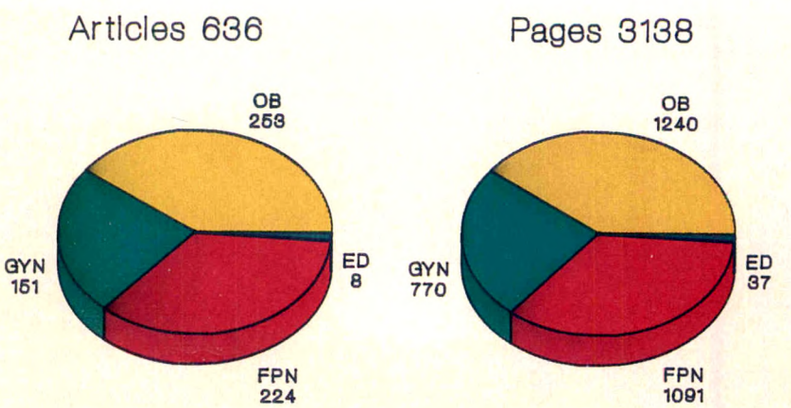


Fig. 2B. Articles and page utilization for 1989-1990 by category.

Clinical Opinion

Maternal serum α-fetoprotein screening: Further consideration of low-volume testing

Daniel M. Strickland, MD,[a] Clifford A. Butzin, PhD,[b] and Frank H. Wians, Jr., PhD[c]
San Antonio, Texas

Unrecognized assay drift that may occur during low-volume (fewer than 500 specimens per week) maternal serum α-fetoprotein testing could result in either underestimation or overestimation of the number of pregnant women who are at increased risk of fetal malformations and genetic anomalies. Quality control software programs that incorporate the use of a multirule Shewhart chart are designed to detect assay drift. Careful selection of quality control sera for inclusion in analytic assays and appropriate application of a multirule quality control procedure to values that are obtained on these control materials should detect assay drift, regardless of the volume of patients' specimens in the run. (AM J OBSTET GYNECOL 1991;164:711-4.)

Key words: Maternal serum α-fetoprotein, low-volume maternal serum α-fetoprotein testing, neural tube defects, quality control

Since 1983, when the Food and Drug Administration first approved the use of α-fetoprotein kits for the detection of open neural tube defects, universal maternal serum α-fetoprotein screening for open neural tube defects, chromosomal anomalies (e.g., Down syndrome), and other congenital birth defects has expanded rapidly in the United States.[1, 2] The use of maternal serum α-fetoprotein concentration in screening for Down syndrome has expanded despite the fact that none of the commercially available kits for the performance of quantitative measurement of α-fetoprotein concentration are currently approved by the Food and Drug Administration for investigation of Down syndrome, and no kits are in the process of being reviewed for approval.[3]

Several aspects of maternal serum α-fetoprotein screening are controversial, such as cost-effectiveness, given the low prevalence of fetal open neural tube defects (1 to 2 cases per 1000 births) in the general obstetric population in the United States[4, 5]; weight correction of multiple of the median values[6-8]; use of a multiple of the median cutoff to investigate Down syndrome[9-14]; reporting of risk data for open neural tube defects, ventral wall defects, and Down syndrome[15, 16]; and the quality of maternal serum α-fetoprotein results from laboratories that perform low-volume testing.[17, 18] Macri et al.[17] have suggested that the quality of maternal serum α-fetoprotein test results that come from small, decentralized laboratories may be less than optimal because of the inability to identify and control assay drift with low-volume (i.e., <500 specimens per week) maternal serum α-fetoprotein testing. Interestingly, 64% of all laboratories that participated in the Maternal AFP [α-fetoprotein] Proficiency Survey (Set FP-D, 1989), which was administered by the American College of Pathologists, indicated a maternal serum α-fetoprotein volume of ≤50 specimens per week (Table I).

Assay drift occurs when analytic bias or imprecision causes a systematic change in assay performance, such that results are not accurate for analyte concentration that is obtained with an assay in which drift has occurred. In the case of maternal serum α-fetoprotein testing, unrecognized assay drift could result in either underestimation or overestimation of the number of pregnant women who are at increased risk of fetal malformations and genetic anomalies.[17]

Assay drift, in the context as discussed by Macri et al.,[17] can be either positive or negative, depending on whether the value for the mean multiple of the

From the Department of Obstetrics and Gynecology/SGHO,[a] Clinical Investigation Directorate/SGS,[b] and Department of Pathology/SGHLCC,[c] Wilford Hall United States Air Force Medical Center, Lackland Air Force Base.
All views and opinions herein are those of the authors and do not necessarily reflect those of the Department of the Air Force or the Department of Defense.
Reprint requests: Dr. Frank H. Wians, Jr., Department of Pathology, Wilford Hall USAF Medical Center/SGHLCC, Lackland AFB, TX 78236-5300.
6/1/24104

Table I. Number of α-fetoprotein analyses performed per week by laboratories that participate in a maternal α-fetoprotein proficiency survey*

α-Fetoprotein analyses (No./wk)	*No. of labs*	*% of total*
<25	74	40.2
25-50	44	23.9
51-100	33	17.9
>100	33	17.9
TOTAL	184	99.9

*Data from Participant Summary, Maternal AFP Screening Survey, Set FP-D. Northfield, Illinois: College of American Pathologists, 1989.

median for all patients' samples in an individual maternal serum α-fetoprotein run or a series of runs (e.g., all runs during a 1-month period) lies above (positive drift) or below (negative drift) the limits of the 95% confidence interval, which was previously established with multiple of the median values of a large population. Low maternal serum α-fetoprotein weekly volume (i.e., <100 patients' specimens per assay, run five times per week) can result in a >47% probability that 10% assay drift (either positive or negative) will be undetected.[17] The solution to this problem that Macri et al.[17] propose is to establish regional programs for maternal serum α-fetoprotein testing with sufficient test volume to permit identification and control of assay drift.

If only patients' data from a particular analytic run are used to assess the presence or absence of assay drift, then estimation of the magnitude of type II error in the detection of assay drift involves the application of power analysis. A type II error would result in failure to detect assay drift, whereas a type I error would result in false identification of assay drift. Currently available quality control software programs are capable of detecting both of these types of errors.[19-21]

Most statistics textbooks (such as that of Winer[22]) refer to three determinants of the magnitude of type II error that are germane to the calculation of assay drift: the level of significance (i.e., type I error) that is used in hypothesis testing, sample size, and sample variance. The acceptable level of type I error is the most easily manipulated of these parameters because it is designated by the investigator before the hypothesis is tested. In general, however, if the magnitude of allowable type I error is decreased, then the type II error rate is increased.

Macri et al.[17] used a level of significance of 0.05 to develop the data that are presented in Table I of their article. If this level were to be relaxed to 0.10, the ability to detect assay drift would be improved significantly, but it would be at the expense of increased probability of false identification.

Macri et al.[17] discuss only the effect of sample size on the probability that assay drift will be undetected. Sample variance is equally important, however, because the lower the variability of assay results, the narrower the confidence interval around the mean multiple of the median that is determined from a large number of multiple of the median values that are obtained with this assay, and consequently, the probability that significant assay drift will be undetected is lower.

Although Macri et al.[17] do not provide data on the variance that was observed in their population of multiple of the median values, we estimate from their data that the ratio of their population mean (u) to the population standard deviation (s) was 1.7. With the use of multiple of the median data (n = 1406) from our laboratory over an 11-month period (two maternal serum α-fetoprotein runs per week or approximately 32 specimens per week), we obtained these values: u = 1.123, s = 0.485, and u/s = 2.32.

Thus data that were obtained from our laboratory with the Tandem-E α-fetoprotein assay (Hybritech Inc, San Diego, Calif.) to quantitate maternal serum α-fetoprotein levels and to establish reference range median maternal serum α-fetoprotein values for 15 to 20 weeks' gestational age in our obstetric population are less variable than those that are presented by Macri et al.[17] With our data for population mean, standard deviation, population mean divided by standard deviation and a specimen volume of fewer than 30 patients' samples per assay, we calculated an 18% probability of missing 20% assay drift (either positive or negative), whereas at this specimen volume, Macri et al.[17] calculated a >38% probability of missing 20% assay drift. More importantly, Best[23] has shown that an improvement in assay precision results in higher detection efficiency for open neural tube defects, fewer false-positive results, a reduction in the number of follow-up procedures, and an overall decrease in the cost of maternal serum α-fetoprotein testing to the patient.

Hullin et al.,[24] who routinely perform assays for maternal serum α-fetoprotein concentration for a minimum of 120 patients' samples per week, have suggested that patients' data plotted in CUSUM (cumulative sum) form can also provide an early warning of potential problems in the maternal serum α-fetoprotein screening program. Thus assay drift can be detected, even with relatively low-volume maternal serum α-fetoprotein testing.

Moreover, commercially available quality control software programs, which are routinely used by many clinical laboratories, incorporate the use of a multirule Shewhart chart to reduce the probability of false re-

jection of a run when the analytic method is in fact stable and to increase the probability of error detection.[19-21] The types of errors that these quality control programs are designed to detect include assay drift, which causes a systematic analytic error, and an increase in the standard deviation, which causes an increase in the random analytic error of the method.[20] Thus careful selection of quality control sera for inclusion in analytic assays and appropriate application of a multirule quality control procedure to values that are obtained on these control materials should detect assay drift, regardless of the volume of patients' specimens in the run.

High-volume maternal serum α-fetoprotein testing, however, as pointed out by Marci et al.,[17] is usually more cost-effective, especially when immunoassay methods that require several calibrator and control samples with each assay are used. In addition, centralization of maternal serum α-fetoprotein testing in a few regional centers that specialize in providing patient-specific risk data that are relevant to open neural tube defects, ventral wall defects, Down syndrome, and other chromosomal anomalies is likely to enhance the quality of the information that is provided by these centers as compared with that provided by small laboratories with a much smaller maternal serum α-fetoprotein data base and larger workload concerns.

Recently, we and others have advocated centralized maternal serum α-fetoprotein testing among military medical treatment facilities by establishing regional centers to perform this testing.[25, 26] It is our opinion that the advantages of regional maternal serum α-fetoprotein screening programs go beyond simply increasing specimen volume sufficiently enough to be able to identify and control assay drift.

The cost of maternal serum α-fetoprotein testing by commercial reference laboratories can vary between $18 and $45 per test, compared with approximately $9 per reportable patient's result in our laboratory.[26] Thus we achieved a savings of $9 to $36 per maternal serum α-fetoprotein test. We have suggested previously that this savings could be increased further, if regionalized maternal serum α-fetoprotein testing were to be established for all Department of Defense medical treatment facilities.[26]

Thus high-volume maternal serum α-fetoprotein testing may be desirable more on the grounds of cost-effectiveness and consolidation of clinical data than for detection of assay drift. Until regional centers are established for performance of this testing, however, the application of stringent quality control practices and adherence to the guidelines that have been suggested by Milunsky and Haddow[27] and by Knight et al.[2] for evaluation of the quality of maternal serum α-fetoprotein screening programs will increase the probability of obtaining accurate and reliable results in laboratories that perform low-volume maternal serum α-fetoprotein testing.

As pointed out by Dr. H.B. Soloway,[5] "FDA should not be concerned with how many [maternal serum α-fetoprotein] tests a lab performs, but rather how often it gets the right result. . . . The FDA notion that bigger is better is as fallacious in respect to laboratory testing as it is in respect to Federal bureaucracies."

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Posterior colporrhaphy and perineorrhaphy: Separate and distinct operations

David H. Nichols, MD
Providence, Rhode Island

Posterior vaginal repair is often poorly understood and ineffectively performed. The goals of reconstructive surgery emphasize relief of symptoms and restoration of normal anatomic relationships and of function. A useful method to preoperatively determine posterior vaginal wall weakness is outlined along with a surgical description of important steps in surgical reconstruction. (Am J Obstet Gynecol 1991;164:714-21.)

Key words: Posterior colporrhaphy, rectocele, perineorrhaphy, defective perineum, posterior vaginal repair

Posterior repair is generally among the most misunderstood and poorly performed common gynecologic surgical procedures. Some of the dissatisfaction relates to the failure to relieve the patient's referable symptoms and the production of significant postoperative dyspareunia.

Although rectocele is fairly common among multiparous women, there is considerable confusion and divergence of opinion concerning not only the related symptoms and indications for repair, but also the anatomic goals sought by the repair and therefore the specific techniques of repair used to achieve them. Rectocele and perineal defect are anatomically separate, but they may coexist (Fig. 1). Each may be repaired when symptomatic, but by quite separate and distinctive surgical procedures. Rectocele is fundamentally a defect of the vagina and its supports, not of the rectum. The symptoms of rectocele are primarily inability to completely empty the bowel, often requiring manual vaginal or perineal expression to complete evacuation and often followed by postevacuation rectal discomfort, probably related to venous congestion consequent to straining. Constipation, per se, is not a symptom of rectocele, although it may coexist. There are many constipated women without rectocele, many women with rectocele who are not constipated, and some women with constipation who have rectocele. Surgical repair of rectocele will not necessarily relieve constipation, the latter being more likely a disorder in bowel habits and function, but not necessarily of anatomy.

A gaping perineum, a result of perineal defect, may at times subtract from coital satisfaction, and this may be improved by perineorrhaphy. The latter will not convert or correct a marital discomfort that is a result

From the Department of Obstetrics and Gynecology, Brown University Program in Medicine, Women and Infants Hospital.
Reprint requests: David H. Nichols, MD, Department of Obstetrics and Gynecology, Women and Infants Hospital, 101 Dudley St., Providence, RI 02905.
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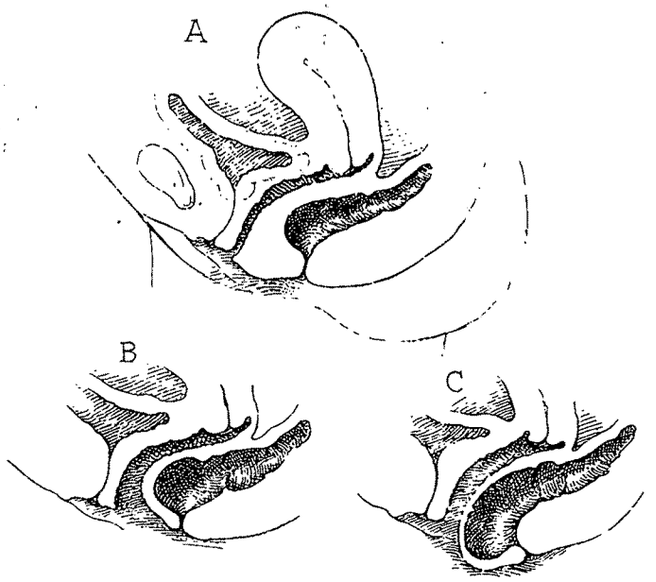


Fig. 1. A, Normal relationship between vagina, perineum, and rectum is indicated. **B,** Perineal defect is noted, but there is no rectocele. **C,** There is both perineal defect and rectocele, and perineorrhaphy should be accompanied by appropriate posterior colporrhaphy. (From Nichols DH, Randall CL. Vaginal surgery. 3rd ed. Baltimore: Williams & Wilkins, 1989:271. By permission.)

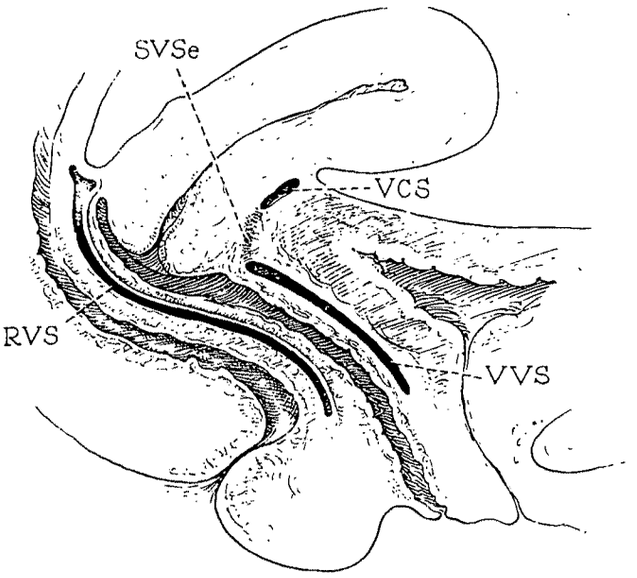


Fig. 2. Rectovaginal space *(RVS)* is shown between rectum and vagina, extending from perineal body to bottom of cul-de-sac of Douglas. Rectovaginal septum is a condensation of tissue attached to underside of posterior vaginal wall along with full length of rectovaginal space. Supravaginal septum *(SVSe)*, vesicocervical space *(VCS)*, and the vesicovaginal space *(VVS)* are shown. (From Nichols DH, Randall CL. Vaginal surgery. 3rd ed. Baltimore: Williams & Wilkins, 1989:34. By permission.)

of other unrelated interpersonal dissatisfactions or conflicts. The goals of reconstructive surgery are relief of symptoms, restoration of anatomy, and restoration of function.

The normal anatomy of the posterior vaginal wall is shown in Fig. 2. The "S-shaped" curve of the vagina ends just in front of the hollow of the sacrum. The vagina is separated from the rectum by the avascular

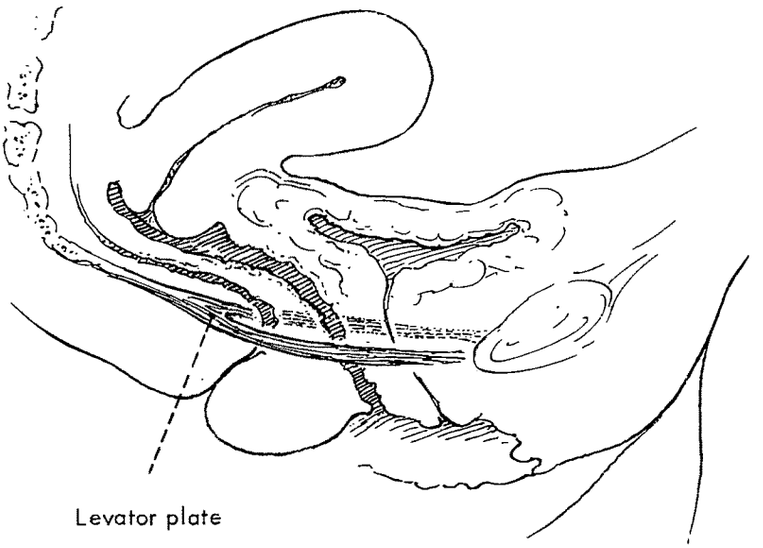


Fig. 3. Normal axis is shown. Notice the almost horizontal upper vagina and rectum lying on and parallel to levator plate. Latter is formed by fusion of pubococcygei muscle posterior to rectum. (Reprinted with permission from The American College of Obstetricians and Gynecologists. Nichols DH, Milley PS, Randall CL. Significance of restoration of normal vaginal depth and axis. Obstet Gynecol 1970;36:251-6.)

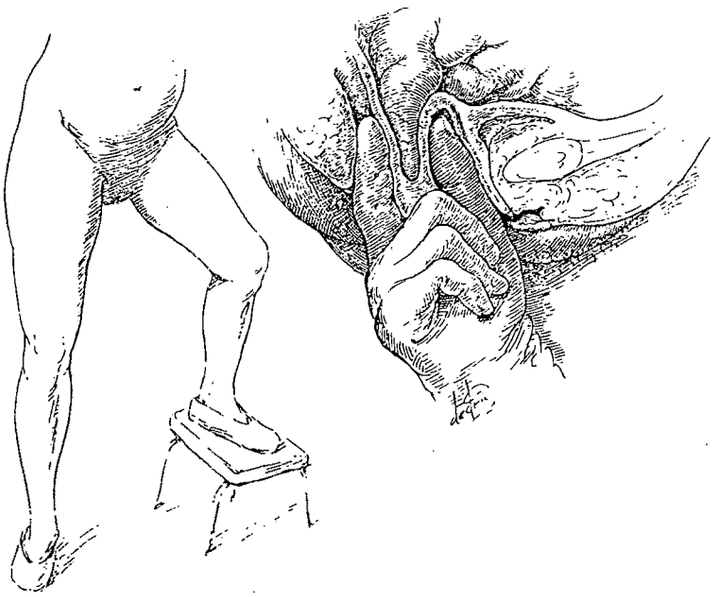


Fig. 4. Examination of patient in standing position permits thumb in vagina to note and replace any descent of vaginal vault while index finger introduced into rectum permits evaluation of any possible rectocele. When patient strains, any enterocele present is evidenced by palpation of bowel-filled sac dissecting the rectovaginal septum. (From Nichols DH. Repair of enterocele and prolapse of the vaginal vault. In: Barber H, ed. Goldsmith's practice of surgery. Danbury, Connecticut: Cine-Med. Copyright 1981, Cine-Med. By permission.)

rectovaginal space, which permits the rectum and vagina to function independently of one another and therefore should be preserved after reconstructive surgery. There is a thin membrane-like connective tissue called the fascia of Denonvilliers that is fused to the underside of the posterior vaginal wall.[1] It extends downward from the bottom of the cul-de-sac of Douglas to its attachment to the upper margin of the peri-

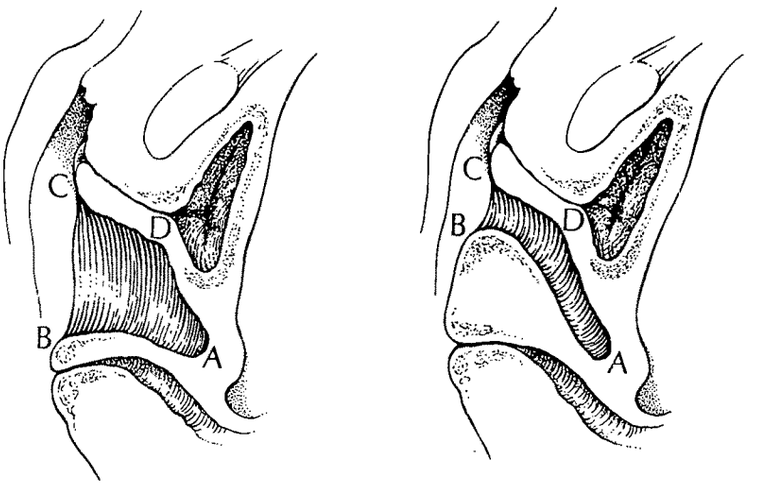


Fig. 5. Effect of perineorrhaphy on lengthening the posterior vaginal wall is shown. Sagittal drawing of pelvis of patient with defective perineum is at *left.* Anterior vaginal wall *(ADC)* is longer than posterior wall *(AB).* Lengthening of posterior vagina *(AB)* after perineorrhaphy is shown at *right.* Length of anterior vaginal wall *(ADC)* is unchanged. (From Nichols DH, Randall CL. Vaginal surgery. 3rd ed. Baltimore: Williams & Wilkins, 1989:293. By permission.)

neal body. When this caudal attachment is avulsed, the perineum is destabilized, and such weakness here is one cause of low rectocele. Reattachment should be achieved surgically. The fascia of Denonvilliers has sufficient strength that it may be used effectively during posterior colporrhaphy.[2] It represents a peritoneal fusion layer of the obliterated extension of the pelvic cavity and its lining peritoneum that during fetal development extended to the perineal body. Failure of fusion gives rise to a congenital deepness of the cul-de-sac in adult life, and when filled with bowel constitutes congenital enterocele.

Because failure of fusion thins the fascia of Denonvilliers, it also weakens the overlying posterior upper vaginal wall, a weakness conducive to high rectocele and accounts in part for the frequent coexistence of high rectocele with enterocele. So frequent is this association that when one is found the other must be routinely sought and usually repaired at the same time, lest it progress and become symptomatic and require subsequent surgery.

The axis of the upper vagina is more or less horizontal. This is because the upper vagina rests on a usually empty rectum, which in turn lies more or less passively on the levator plate, the latter a fusion of the right and left pubococcygei posterior to the rectum (Fig. 3). When the pelvic diaphragm is intact, it is itself in a horizontally oriented plane. Very few fibers of the pubococcygei decussate anterior to the rectum. The lateral walls of the vagina at its midportion are indirectly attached to the pubococcygei by the connective tissue fibers of Luschka. Therefore techniques of the posterior repair that emphasize primarily a bringing together of the levators between the vagina and rectum are essentially unanatomic. Ridges and painful muscular fibrosis from these levator stitches account for most of the postsurgical dyspareunia or apareunia reported by Jeffcoate.[3] He advised against posterior repair unless significant damage was confirmed by examination of the unanesthetized patient (because anesthesia effectively paralyzes the voluntary muscles of the pelvis, rendering a false idea of anatomic weakness from examination of the anesthetized patient).

Why, then, is there so much use of the Halban-Hegar prerectal levator plication? It must be remembered that in the days before intraperitoneal surgery and hysterectomy became surgically safe, the treatment of symptomatic uterine prolapse was by the wearing of an intravaginal pessary. When, after a time, the genital or levator hiatus became progressively more widened, the patient could not longer retain the pessary and the patient, now older, was worse off than ever. The introduction around the turn of the century of levator plication as a feature of perineorrhaphy enabled the patient to retain a vaginal pessary because the plication narrowed the levator or genital hiatus. Often, the only treatment that could be offered the patient was that of complete obliteration of the vagina as by Lafort colpocleisis.

Pessary use was gradually abandoned when vaginal hysterectomy became safer and a popular treatment of prolapse, and the erroneous concept was embraced that suggested that a firm perineum functioned as a "cork in the neck of a bottle" and that perineorrhaphy itself would retard or prevent uterine prolapse. Kelly[1] had previously observed the untruth of this view, noting

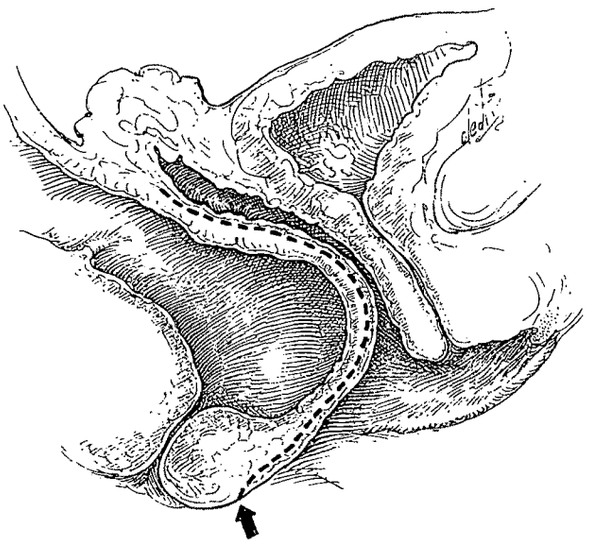


Fig. 6. Sagittal section shows initial line of dissection exposing full perineum and rectocele. Above the perineum, dissection enters rectovaginal space and continues proximal to highest point of rectocele, as indicated by *dashed line.* (From Nichols DH, Randall CL. Vaginal surgery. 3rd ed. Baltimore: Williams & Wilkins, 1989. By permission.)

that uterine prolapse was uncommon among the many women he had seen who had long before suffered unrepaired complete perineal tears.

Rectocele and perineal defect may develop from rapid or overdistention of a poorly elasticized vaginal wall during labor. Such lack of elasticity may be predicted as a feature of aging among primigravid women older than 25 years and among persons with many wide striae that imply a very low elastic index.[5] The risk of rectocele may be lessened for such a patient by early and adequate episiotomy. Weakness of the posterior vaginal wall may also develop coincident to eversion of the vaginal vault, sharing or avulsing the fibers that attach the vagina to the pelvic diaphragm. This displacement of the vagina creates a weakness into which the anterior rectal wall may expand—another cause of rectocele.

Preliminary physical examination of the pelvis of the unanesthetized patient should attempt to find correlation between the patient's symptoms and anatomic disorder. With rectocele the examiner, during rectal examination, can always hook a finger into the pocket in which fecal material may become trapped. When coincident and generally unsuspected but contributory prolapse of the vaginal vault is determined by pelvic examination of the patient while she is standing and straining (Fig. 4), appropriate colpopexy should be included in the surgical plan.

Because the length of the perineal body is the same as that of female urethra, reconstruction of a damaged perineum will improve urethral support and function. It is interesting that perineorrhaphy will also lengthen the vagina by the mechanism shown in Fig. 5. With this in mind, we have for years used an anatomically correct reconstructive technique for posterior colporrhaphy and perineorrhaphy to achieve the goals described. Long-acting synthetic polyglycolic acid-type suture is used throughout, and if postmenopausal the patient is started preoperatively on a course of intravaginal estrogen replacement with an estrogen cream. This is continued for several months postoperatively. It will restore and improve vaginal elasticity, and by increasing the vaginal blood supply and thickening the vagina it promotes better wound healing. If a significant prolapse of the vaginal vault is found during examination, it should be repaired as should any coexistent enterocele.

Dissection and repair of the rectocele should begin at its highest point (Fig. 6). Because the perineal body is shaped essentially like a pyramid with its widest point or base at its bottom, the undersurface of the perineal body is uncovered by a V-shaped incision. The width of the V is determined by the desired size of the introitus at the conclusion of the operation, being careful to leave it a little loose for the aging patient so that it may not constitute a coital obstruction (Fig. 7). The dissection is carried beneath the posterior vaginal wall, separating it from the vaginal surface of the perineal body, until the rectovaginal space is identified and opened. A strip of an appropriate width of full-thickness posterior vaginal wall is removed, sufficient to relieve the rectocele and at the same time leave a normal-sized vagina diameter, one able to admit three fingerbreadths after the conclusion of the procedure. The sides of the posterior vaginal wall are approximated by a single layer of subcuticular sutures that incorporate the fascia

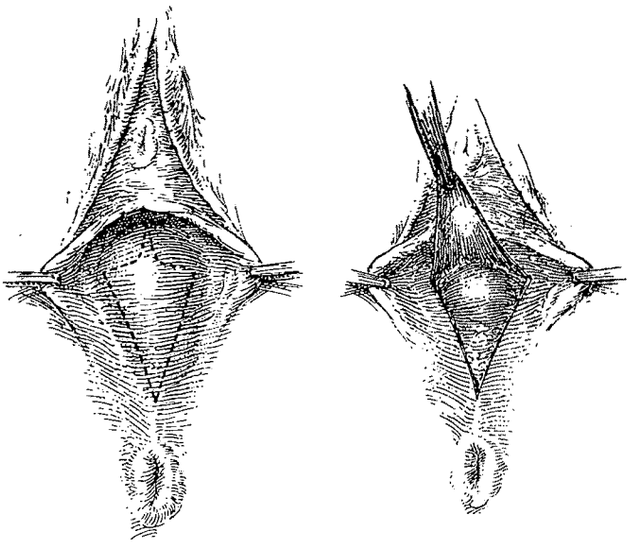


Fig. 7. Diamond-shaped piece of tissue of appropriate width is estimated as indicated in drawing at *left*. Skin is incised and dissection carried superiorly into vagina, exposing full site of future perineal body. If rectocele is present, rectovaginal space is entered, and the dissection freeing rectum from vagina carried to level above any rectocele that is present. (From Nichols DH, Randall CL. Vaginal surgery. 3rd ed. Baltimore: Williams & Wilkins, 1989:288. By permission.)

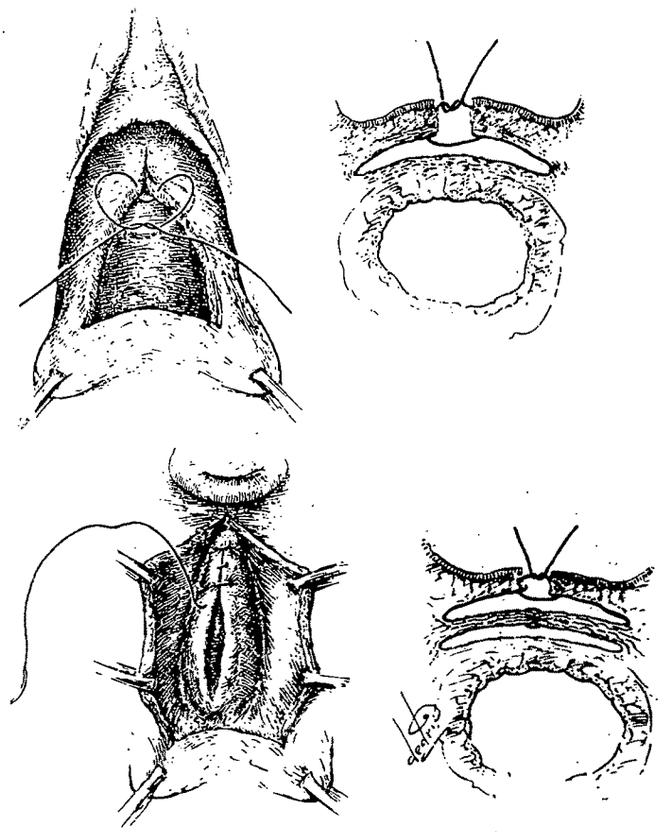


Fig. 8. In the Goff method of posterior colporrhaphy, appropriate full-thickness wedge of posterior vaginal wall is excised and tissues including fused rectovaginal septum are closed from side-to-side as noted in *upper portion* of illustration. Bullard modification is shown *below*, in which rectovaginal septum is dissected from posterior vaginal wall and closed as separate layer between rectum and vaginal membrane. (From Nichols DH, Randall CL. Vaginal surgery. 3rd ed. Baltimore: Williams & Wilkins, 1989:282. By permission.)

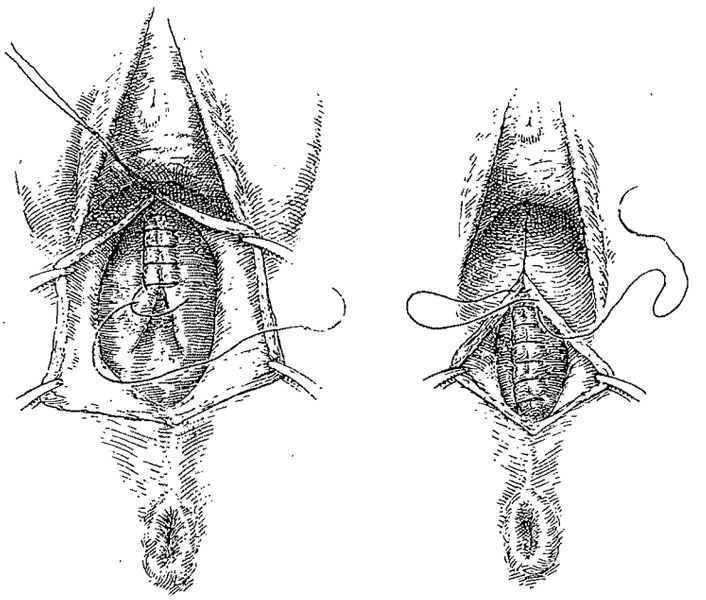


Fig. 9. Any ballooning of anterior rectal wall may be corrected by one or more layers of running or locked absorbable suture commencing proximal to defect and continuing distally for its full length. (From Nichols DH, Randall CL. Vaginal surgery. 3rd ed. Baltimore: Williams & Wilkins, 1989:279. By permission.)

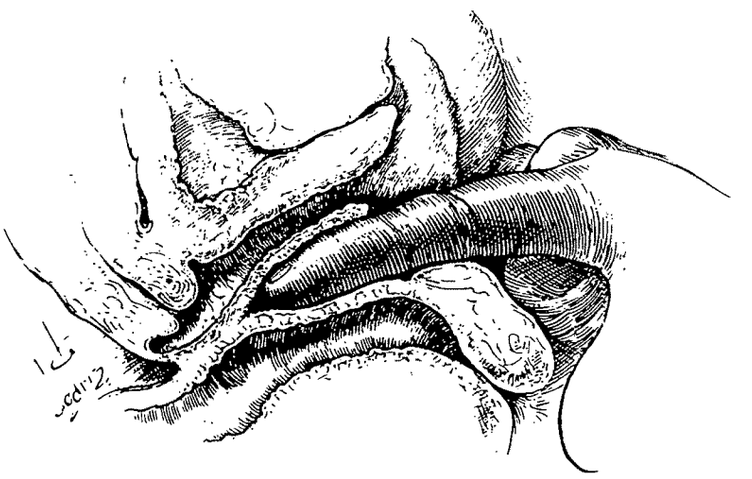


Fig. 10. At completion of posterior colporrhaphy and before starting perineorrhaphy, operator should be able to insert an index finger freely between posterior vaginal wall and anterior surface of rectum, showing desired freedom of this rectovaginal space. (From Nichols DH, Randall CL. Vaginal surgery. 3rd ed. Baltimore: Williams & Wilkins, 1989:283. By permission.)

of Denonvilliers. Alternately the rectovaginal septum (the fascia of Denonvilliers plus a little of the posterior fibromuscular layer of the vagina) may be dissected from the undersurface of the vagina and closed as a separate layer (Fig. 8). Any secondary ballooning of the anterior rectal wall may be corrected by a running locked suture (Fig. 9) extending to the cranial margin of the perineal body. At the conclusion of the procedure and before the perineorrhaphy, the operator should be able to insert a finger into the rectovaginal space to show its freedom (Fig. 10).

The perineal body is reconstructed by a series of horizontal mattress sutures placed in the soft tissues medial to the pubococcygei as shown in Fig. 11. When

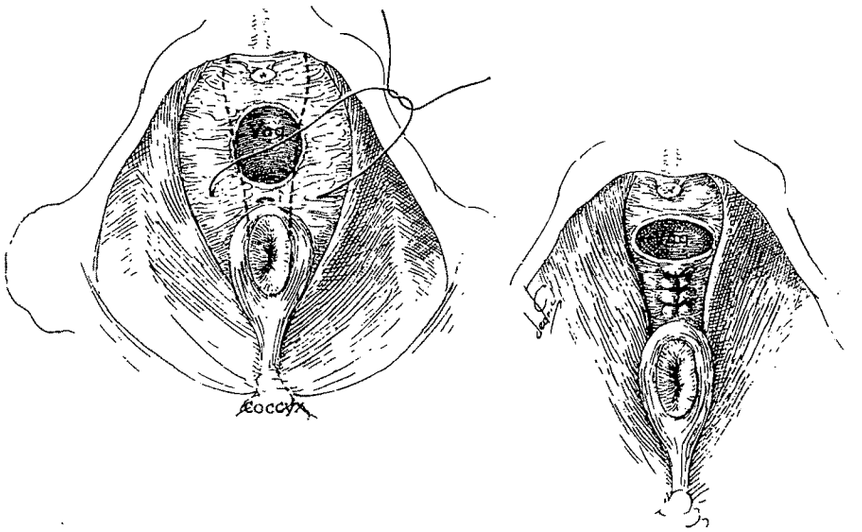


Fig. 11. Perineorrhaphy may be accomplished without placement of stitches directly into muscle bellies of pubococcygei, as shown with wide genital hiatus at *left*. When interrupted stitches in perineal body are tied, lateral attachments of this tissue to fascia of pubococcygei will bring latter closer together, narrowing genital hiatus to new position as noted by *dotted line*. End result is illustrated at *right*. Genital hiatus is effectively narrowed. No stitches were placed directly into muscular substance of pubococcygei. (From Nichols DH, Randall CL. Vaginal surgery. 3rd ed. Baltimore: Williams & Wilkins, 1989:291. By permission.)

these are tied, the levator-genital hiatus will have been effectively narrowed without stitches placed directly into the muscular substance of the pubococcygei. However, levator stitches may be used in the uncommon situation in which there is an extreme perineal defect and no other soft tissues can be found that can be brought together. When levator stitches are used, the operator should check for ridge production after each stitch is placed. If a ridge is found, the stitch is removed and replaced by a stitch located closer to the rectum. Levator stitches should never be tied tightly, lest they destroy the muscle in which they have been placed, converting it to often-tender fibrous tissue that may require future secondary perineotomy for relief. The perineal skin is reapproximated in the midline by a running subcuticular suture. The caliber or width of the vagina is assessed carefully with consideration to future coital satisfaction. If unexpectedly constricted, appropriate full vaginal thickness lateral relaxing incisions are made at the 3 o'clock and 9 o'clock positions. Rectal examination is done to confirm the integrity of the rectum; if a transgressing stitch is found, it is cut on the rectal side, lessening the postoperative pain and risk of rectovaginal fistula, and the suture is allowed to retract.

After surgery, the patient is placed on stool softeners and gentle laxatives to avoid straining at stool against the fresh repair. Ascorbic acid is given for a month or longer to promote wound healing, and postoperative estrogen supplementation is given if the patient is postmenopausal. Coitus may be resumed after 4 to 6 weeks of healing.

We adapted these techniques[6] from the works of our forebearers[7] and have used them with modifications for more than 40 years. They are anatomically and functionally correct, and neither postoperative dyspareunia nor obstructive apareunia has been observed.

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Stratification and standards: A quality assurance perspective

Presidential address

Stanley Zinberg, MD
New York, New York

A diminished public respect for physicians, a decrease in professional autonomy, and an increased regulatory presence have led to extensive changes in medical practice in the past 25 years. Along with rising costs of medical care, there has been a resurgence in interest in the quality of health care, and a considerable body of literature on the monitoring and evaluation of health services has been produced. Quality assurance programs frequently evoke negative and skeptical responses from many physicians for a variety of reasons. Crucial to the potential effectiveness of quality assessment methods is the setting of standards. Physician clinicians should be strongly encouraged to participate directly and seriously in the development of standards. The quality assurance process should be viewed by physicians as an opportunity to recapture the control of the profession and to demonstrate to patients, in an objective manner, the legitimate high quality of care rendered. (AM J OBSTET GYNECOL 1991;164:722-8.)

Key words: Quality assurance, medical practice, standards, stratification

You had to live—did live, from habit that became instinct—in the assumption that every sound you made was overheard, and, except in darkness, every movement scrutinized.

George Orwell, *1984*

The year 1984 has come and gone and, for most Americans, George Orwell's fictional account of a world of totalitarian control appears not to have come to pass. How many of us involved in medical practice would agree? A Rip Van Winkle scenario that uses 1965 to 1990 as the period of hibernation would cause even a casual observer to concede that there have been extensive changes in the way physicians practice medicine.

These changes can be illustrated by listing six reasons why gifted college students chose to apply to medical school in the years since World War II. Headed by an interest in science and biology and the personal satisfaction derived from providing an important service to individuals and society, the future physician was aware of the prestige and respect that accompanied a career in medicine. Professional autonomy was automatically present and financial security and a wide choice of lifestyles were guaranteed.

Interest in the biological sciences and the opportunities to help people, which are the essence of the profession, have not diminished and continue to attract prospective physicians. Expanded medical information permits more effective patient treatment than in the past. However, public respect for physicians has weakened in the last two decades and, although this appears to be part of a general diminished public confidence in all American institutions (of which medicine suffers the least), the adverse impact on the doctor-patient relationship has been disheartening.[1] Professional autonomy is disappearing, and government agencies, insurance companies, and consumer groups question medical decisions and often dictate the course of treatment. Previously, the life-styles of physicians were determined by specialty and geographic location. Currently, regulatory groups are creating guidelines, incentives, and disincentives to control the number of physicians who enter specialities and to resolve a geographic maldistribution problem. Physicians' earnings are influenced by government, insurance companies, and a variety of health maintenance organizations.

In spite of a higher technical sophistication of ser-

From the Departments of Obstetrics and Gynecology, the New York Infirmary Beekman Downtown Hospital and the New York University School of Medicine.
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Received for publication July 5, 1990; revised October 25, 1990; accepted November 6, 1990.
Reprint requests: Stanley Zinberg, MD, New York Infirmary Beekman Downtown Hospital, 170 William Street, New York, NY 10038.
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vices, patients balk at the rising level of physicians' charges, which increase annually at a rate that exceeds inflation. Although these same patients recognize the physician's commitment of resources to the arduous study and practice of medicine, they perceive the health care commodity as an entitlement, and they resent paying for the misery of an illness that they did not request and over which they have no control, especially when they view the fees as excessive. To stoke the fire, a malpractice litigation problem of absurd proportions affects the traditional bond between doctor and patient. Dr. Saul Radovsky[2] wrote that "A final offsetting factor is the [disaffection] of doctors. Having believed themselves to be highly regarded by the public and having considered themselves to be exercising professional and economic responsibility in a calling, they now see themselves cast as wrongdoers and incompetents who yearly require new laws, regulations, admonitions, court decisions and exposés to make them more honest, ethical, competent, corrigible, and contrite. Perhaps it is no wonder if many now look for a change or an exit."

The temptation to link current circumstances of physician discontent to a decrease in the number and quality of medical school applicants is hard to resist. From a historical peak of 42,624 applicants in 1974, the applicant pool had decreased to 26,721 in 1988. The greatest decrease in applications was among white males but, in 1985, it was noted for the first time that the decrease included women and underrepresented minorities.[3] A demonstration of a decrease in quality of the applicant pool is more problematic, and the Medical College Admission Test results showed no significant changes, except possibly in the reading section. In 1981, 3.6% of applicants and 5.6% of matriculants scored 12 or greater, while in 1988 only 2.8% of applicants and 3.7% of matriculants achieved the same score in that section.

The significant event that led to the current situation was the passage in 1965 of the Medicare Act, which provided medical coverage for 20 million Americans and cost $4.5 billion in 1967; by 1985, it covered 30 million aged and disabled individuals and cost $70 billion for a program that provided less coverage than it had 18 years earlier. The central issue is money. In 1977, the nation's health care bill amounted to $160 billion, or 8.6% of the gross national product. In that year, for the first time, health care outstripped the costs for national defense and became the country's third largest industry, after agriculture and construction. In 1990, 13 years later, the nation's health bill is estimated to be $661 billion, which is currently 11.4% of the gross national product.[4] Of this $661 billion, $137 billion (21%) will be for physician services and $252 billion (38%) will be for hospital billings. Government, through its tax revenues, pays for more than 40% of all health care expenditures; the health bill for the federal government alone in 1989 was $114 billion; $24 billion of this was spent in medicare payments to physicians. One can take the view that not enough is being spent on health care and that 11% to 12% of a nation's gross national product spent on health is appropriate. Perhaps so! However, there appears to be little enthusiasm for this view from government, corporate America, or the citizenry at large. The federal government is currently the single greatest consumer of health care services, and it has often been said, "He who pays the piper, calls the tune." And the song we hear played so often goes like this: "Too much is being spent on health care. Does it need to cost so much? How do we know we are getting value for our money?"

This leads me to the principal theme: society's efforts to impose accountability on the individuals and institutions that provide health care services. This process has been euphemistically called quality assurance, and it is my thesis that the way in which physicians respond to the challenges created by this process will determine whether medical practice continues to be an academic, effective, and compassionate calling. We, as professionals, must define our standards, assess the results of our efforts, and judge the quality of our activities. This must not be left to others. Further, the proliferating quality assurance requirements, which at times tend to suffocate us, are probably our best and only opportunity to recapture the control of our profession and to restore the autonomy essential for attracting talented future physicians and for ensuring the best patient care.

The resurgence of interest in the quality of health care stems from increased government spending, rising costs of medical care, and rapid advances in medical science. There is agreement that the current interest in quality is largely finance driven, and it would be fantasy to believe that government and industry would be as preoccupied with quality level if these services were inexpensive. However, there are other considerations that stimulate this interest, such as instances of demonstrated poor level of care, increased consumer expectations, a rise in malpractice suits, and the proliferation of the variety of service institutions in which medical care is provided.

Historically, two basic principles of quality assurance were attributed to Hippocrates and Maimonides. Hippocrates wrote: "When there are procedures which can be right or wrong, a consideration of these must constitute a science. There is no science when there is neither a right way or wrong way." In 1198 AD Maimonides wrote the forerunner of the second opinion: "One should know that even if attended by a well-known physician, or several physicians, he should not submit, and take the strong remedies except on the advice of a physician who is very accomplished, whose knowledge has been verified, and whose experience has been attested."

In the early 1900s the Clinical Congress of Surgeons of North America developed a system for the evaluation of hospitals. The American pioneer in quality assurance was a Boston surgeon, Dr. Ernest Amory Codman,[5] who influenced the Clinical Congress of Surgeons to implement his method of end results analysis in its evaluation of hospitals. In 1916 he described issues similar to those used today to evaluate quality of surgical care, which included licensure and certification of physicians, accreditation of institutions, severity and stage of disease, comorbidity, patient behavior, and economic barriers to care. Six years before his death, in a largely autobiographic preface, he wrote: "I have suffered from a sense of isolation, because I have always been thinking, or saying, one thing or another, with which other doctors did not agree."

The American College of Surgeons, which was founded in 1913, inaugurated its Hospital Standardization program in 1918, and the concept of hospital accreditation was promoted as a means of assuring good care. Codman's exacting end-result methodology was set aside, and, instead, the American College of Surgeons published the "Minimum Standard," a one-page document, which did not assess outcomes of care but rather addressed structural measures of quality such as adequacy of the physical plant, credentials of the medical and nursing staff, completeness of medical records, and facilities for laboratory and radiologic diagnosis. The first survey resulted in the approval of only 89 of the 692 hospitals (12.9%) surveyed but, by 1950, 94.5% of the hospitals qualified for accreditation.

With the growth of nonsurgical specialities after World War II, the American College of Surgeons joined with the American Medical Association, the American Dental Association, the American Hospital Association, and the American College of Physicians to form the Joint Commission on Accreditation of Hospitals. Because of the increase in the variety of service institutions providing health care, the Joint Commission on Accreditation of Hospitals has recently renamed itself the Joint Commission on Accreditation of Healthcare Organizations (JCAHO). From the one-page "Minimum Standard" of the American College of Surgeons grew the current 334-page volume entitled the *1990 Accreditation Manual for Hospitals*.[6] Although JCAHO accreditation is not mandatory, the process has a quasipublic role because accredited residency programs can only be conducted in JCAHO-approved institutions. Furthermore, the Medicare program uses the JCAHO accreditation as evidence that hospitals comply with the described standards. The new JCAHO standard requires that every hospital have a written quality assurance plan and that "there must be an objective and systematic monitoring and evaluation of the quality and appropriateness of patient care and a pursuit of opportunities to improve patient care and resolve identified problems." In its widely publicized "Agenda for Change," JCAHO is developing a new accreditation process with a uniform set of indicators of patient care quality for specific clinical and organizational units, which will be collected from each hospital and incorporated into a national data base. The pilot phase of this project was initiated with the American College of Obstetricians and Gynecologists. Obstetrics was chosen for several reasons. First, our discipline has a long history of involvement in outcome-type quality assessment activities, which are exemplified by the work of the maternal mortality and perinatal review committees. Second, the college has published a number of quality-assurance-related books over the past 15 years, and third, some believe it is easier to define aspects of care in obstetrics than in other medical specialities.[7]

In 1966, Avedis Donabedian[8] proposed that quality of health services could be monitored by three different approaches, which he termed structure, process, and outcome. Structure included items such as the number of physicians on staff and their qualifications, the nurse-patient ratio, and the equipment and number and size of facilities. These are indirect measures of outcome. The process activity comprised the evaluation of the specific steps of diagnostic and therapeutic procedures actually provided to patients. Most medical audits fall within this category. The outcome component consisted of the results achieved for the patient; it was more difficult to measure because outcome was often temporally removed from the office visit or hospitalization. However, outcome analysis or, as Codman[5] called it, "end-result assessment" is the current focus of the quality assurance requirements of all regulatory and accrediting agencies. The legal responsibility for the establishment of quality assurance programs and the quality of patient care has been assigned to hospital governing bodies.

A review of the quality assurance literature unavoidably raises the perplexing question of the meaning of quality. Although most physicians believe that differences in the quality of care can be instinctively distinguished, our perspectives, predictably, tend to be narrow because we view health as simply the absence of disease. One useful definition of quality is simply adherence to a standard. The brevity of this definition makes it attractive, but it is precisely this attribute that leaves it open to criticism. With a lofty standard, little will be acceptable, and if the standard is minimal, it will fail to discriminate among widely different levels of care.

A number of academicians have pursued the definition of quality. Avedis Donabedian[9] defined quality

as a difficult-to-identify property that medical care has in varying degrees and which, even if identified, requires translation into criteria and standards that can be used in making consistent judgments. Furthermore, he stated that judgments of quality are made not only about the medical care itself but indirectly about the persons who provide care and about the settings or systems within which care is provided. He divided the concept of management into technical care, which is the application of science and technology of medicine, and interpersonal care, which is the management of the social and psychological interactions between doctor and patient. The first represented the science of medicine; the second, the art of medicine. A third element, the amenities, was included under the interpersonal relationship category because it signified concern for patient satisfaction. Donabedian concluded by adopting the view that assessment of quality varies depending on the outcomes sought, the values placed on the outcomes, and the appropriateness of the means used to attain those outcomes. He cautioned that judgments vary depending on whether the assessment is made on behalf of individual patients or the community, whether preferences of individuals are taken into account, and whether monetary cost is included in the assessment. It is clear that for Donabedian, assessment of quality depends heavily on the perspectives of the individuals who make judgments and on the characteristics of the criteria and standards used in those judgments.

A second definition of quality is attributed to Brook and Williams,[10] who described the following formula: Quality of health care = Technical care + Art of care + Technical care × Art of care + E (random error). Technical care represents the adequacy of the diagnostic and therapeutic process, art of care relates to the psychosocial and environmental impact, the interactive term underscores the idea that technical care and art of care are not simply additive, and a random error factor signifies that some outcomes may be determined by chance.

An operational definition of quality presented by William F. Jessee[11] consists of the three following interrelated objectives: (1) optimal achievable benefits of health care for each patient, within the biologic constraints imposed by the person's illness, age, or underlying chronic diseases and within the limitations imposed by the individual's own choices in complying with health care recommendations; (2) minimal expenditure of resources necessary to achieve these benefits; and (3) avoidance of injury or additional disability that results from the provision of health care services. This definition, like that of Donabedian, links benefits, risks, and volume or costs of services. In spite of the intellectual appeal of Jessee's operational definition of quality, even he wondered whether the inclusion of an emphasis on containing costs might provide resistance and controversy in the health professions.

The words "quality assurance" evoke a negative response from many physicians. I call the following reasons, which are most often cited for this resistance, the "Ten Denouncements."

1. *The quality assurance process requires an excessive time commitment.* Even with computer-assisted data collection, the quality review process is time-consuming. It seems that the number of formal and informal meetings generated by these activities increases exponentially.

2. *No evidence exists that quality assurance programs improve quality of care.* The little evidence that exists to support the idea that programs of professional peer-review can lead to changes in medical practice comes from an evaluation of the national Professional Standards Review Organization program by the Health Care Financing Administration in 1978.[12] A number of instances were noted in which improvement in compliance with process or outcome criteria followed Professional Standards Review Organization medical audits. However, no hard data indicate that these activities improve quality. On the other hand, there is a "big-brother" mentality that expresses an attitude that when one is being watched (as in peer review), one tends to be more careful. Nevertheless, agencies insist that we demonstrate an improvement in quality for their invested dollars. This may be comparable to demanding proof from religious institutions that their tax exemptions result in the decrease in the prevalence of sin.

3. *Quality assessment has always been part of medical practice.* Quality assessment and quality assurance are not synonymous. Quality assessment means measuring the level of quality without an effort to change or improve the level of care; quality assurance includes the measurement of the level of care but implies actions to eliminate substandard performance and to improve the effectiveness of treatment. For example, our traditional educational activities of assigning responsibility, delineating scope and important aspects of care, and collecting, organizing, and evaluating data encompass most of the first seven steps of the JCAHO 10-step monitoring and evaluation process. The final three steps (taking action to resolve identified problems, assessing these actions and documenting improvement or lack of improvement, and communicating this information to parties outside our department) are activities with which physicians are uncomfortable. A frequent response from physicians when they are pressured into regulatory activities is that if they had wished

to be policemen, they would have pursued an alternate career. However, these activities, although sometimes distasteful, are implicit in the term "assurance" and are a part of the contemporary expectation of accountability.

4. *The current method of quality assurance has a punitive orientation.* Physicians are concerned with the activity of the Peer Review Organization in quality care assessment. Current federal draft regulations authorize the PRO to deny payment to a physician or hospital for services determined to be of substandard quality. Standards utilized by the local peer review organization are based on fairly general federal guidelines. Sanctions may be imposed on individual physicians by the Peer Review Organization for designated quarterly point totals; these sanctions range from educational intervention to notification of state licensing agencies and professional associations. Patients are notified of denial of payment with the language that the medical care reviewed was not acceptable under the Medicare program. The number of Peer Review Organization citations for quality will increase because of mounting pressure to increase the number of payment denials.

5. *The present system of quality assurance leads to decreased physician autonomy.* Currently in New York State, in addition to JCAHO surveys, the regulatory bodies that affect the quality of patient care include the Accreditation Council for Graduate Medical Education with its residency review surveys, the Medicare Title XVIII reviews, Medicaid reviews, and Peer Review Organization activities. In addition to the New York State Department of Health Article 28 surveys, quality is influenced by the State Board of Professional Medical Conduct, the Impaired Physicians Committee, and the New York State education law and incident reporting regulations, as well as the New York State graduate medical education regulations. These forces affect the physician who is primarily responsible for the quality of patient care.

6. *The existing process of quality assurance promotes unprofessional "cookbook" medicine.* Physicians frequently respond negatively to systematic efforts to develop standards of care because of a concern that medical practice may be reduced to inflexible rules that will prevent physicians from exercising clinical judgment and will stifle independent thinking. Rigid protocols fail to reflect the myriad possibilities that may be encountered in working with real patients.

7. *Quality assurance activities increase the cost of medical care.* Costs associated with the many programs that assure quality have not been accurately measured. It has been suggested that a standard for hospital direct costs be 1% of the operating budget of the institution. If this were a reality, more than $2.5 billion would be spent in 1990 by hospitals alone on quality assurance activities. This figure does not include the monetarily uncompensated time and effort of physicians. In addition, quality assurance committees frequently do not achieve their goals because tangential or trivial issues are addressed at considerable cost. Total costs of these activities are elusive for several reasons. First, difficulty exists in estimating what proportion of an employee's time is spent on quality assurance, except in those instances in which the employee's full-time effort is in this area. Second, since hospitals are theoretically reimbursed for quality assurance activities, finance officers are often reluctant to disclose the exact level of actual spending, and third, indirect costs are difficult to measure. Finally, cost reduction efforts generally contain quality assurance components. David Himmelstein,[13] who explored the issue of administrative waste that he termed "cost without benefit," points out that most cost containment programs "have involved . . . hiring more administrators to ensure delivery of less care . . .."

8. *Quality cannot be defined.* The concept that "quality is in the eye of the beholder" has been used by physicians as a reason that quality assurance programs have not been implemented. This quotation from Donabedian[14] is on target: "Quality is now a term too easily bandied about, and there is little hesitation in proposing that quality can be measured . . .. But this mood of almost belligerent confidence is perhaps premature, for there is much about the concept of quality that is elusive, undefined and unmeasured."

9. *Clinical uncertainties do not lend themselves to evaluation by inflexible quality assurance plans.* David Eddy[15] states that uncertainty "creeps into medical practice through every pore" and that physicians can do more to admit the existence of uncertainty, both to themselves and to their patients.[15]

10. *Fear of public disclosure and legal liability discourages optimal participation in quality assurance activities.* Two examples that breach peer-review confidentiality are the current ability of the Peer Review Organization (PRO) to publicly identify practitioners involved in Medicare or Medicaid claims that are denied for substandard care and a recent Georgia judicial decision that mandates public release of Joint Commission reports. The Health Care and Quality Improvement Act of 1986, which is known as the medical shield law, affords considerable protection to members of hospital staffs who act in good faith to monitor physicians for violations of quality of care. However, the removal of the exemption of the learned professions from antitrust laws has diminished the legal protection of the peer-review process.[16]

The existing bureaucratic programs of quality assurance will continue to exist, as will a demand for intensification of quality assurance activities. The most critical component of the current process is the setting

of standards, and for this reason the profession should be alerted to the fact that, according to Mark R. Chassin,[17] what is new in the emerging debate on methodology to establish standards is "the clear inference that standards of care are too important to be left to physicians to develop and implement." I hope that this is a minority point of view and that the public sector will continue to rely on physicians to set standards. But to which physicians will they turn?

Sociologist Eliot Freidson[18, 19] describes an increasing stratification of the medical profession that consists of an elite group of administrators, educators, and researchers and, below them, the practitioners, who are in the majority. Whereas the elite are the managers, judges, and employers, the practitioners are the managed, the judged, and the employed. This stratification appears to be more important in the segmentation of the profession because it cuts across all specialties and provides the economic, administrative, and cognitive authority over everyday practice. There has been an accelerating increase in the number of physicians who devote their efforts solely to administration. By illustration, the American College of Physicians Executives had 4898 members as of Dec. 31, 1989, and has demonstrated rapid growth. Will government and legislators turn to a cadre of administrative physicians, many of whom have little or no experience in clinical care, to assist them in specifying standards? That is possible because to regulatory agencies one doctor is much like another. But that action would definitely be unfortunate. It would be no more sensible if those agencies sought either a medical school dean who lacked clinical practice experience or a comparable full-time researcher who had never treated patients. Physicians who are involved in patient care (particularly those who are the academic and clinical leaders of our discipline) have the obligation to participate in the standard development process. The decisions in the process cannot be left to nonclinicians, whether they do or do not have medical degrees. Only clinical practitioners have the insight and experience to set these standards.

Different observers view the quality assurance process with varying goals and perspectives. Physicians often see it as interference. Consumers of health care expect quality assurance plans to assess the ability of physicians to make accurate diagnoses and institute appropriate treatments. A hospital administrator may judge a plan to be effective if it focuses on delivering the greatest number of services at the lowest possible institutional cost. The Health Care Financing Administration sees the monitoring of health care as an opportunity to improve medical practice by the collection and distribution of information on health outcomes. The Peer Review Organization measures effectiveness by achieving the greatest number of payment denials, and the New York State Department of Health is on record that these programs will weed out bad doctors and will thereby diminish malpractice. The Joint Commission would be satisfied if all hospitals met its quality assurance standards, and some economists prefer a free-market determination of quality. Certain members of congress see peer-review activities as being one road to dealing with medical malpractice.

In conclusion, consider the following: As part of the new competition, for-profit interests threaten to dominate health care. Free-standing clinics, surgicenters, emergicenters, and urgicenters are springing up all around us, and are directed by medical entrepreneurs who keep score on patient care by counting dollars. As David M. Kinzer[20] has suggested, these businesses market increased utilization and often underprice their services for those people who don't really need them in the first place. In my opinion, however, most patients, when it comes to the issue of their own health, will opt for quality if they can recognize it. The current quality assurance initiatives permit us to accept the challenge of the new competitive environment by giving us the opportunity to demonstrate to our patients that our teaching hospitals and their medical staffs do provide better quality care. If we believe this is so, we will have to prove it!

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Antepartum cultures for *Ureaplasma urealyticum* are not useful in predicting pregnancy outcome

J. Chris Carey, MD,[a, b] William C. Blackwelder, PhD,[c] Robert P. Nugent, PhD,[a] Marie A. Matteson, MS,[d] A. Vijaya Rao, PhD,[d] David A. Eschenbach, MD,[e] Molly L.F. Lee, RNC,[e] Philip J. Rettig, MD,[b] Joan A. Regan, MD,[f] Kim L. Geromanos, RN,[f] David H. Martin, MD,[g] Joseph G. Pastorek, MD,[g] Ronald S. Gibbs, MD,[h] Kathleen A. Lipscomb, RN,[h] and the Vaginal Infections and Prematurity Study Group*

Bethesda, Maryland, Oklahoma City, Oklahoma, Research Triangle Park, North Carolina, Seattle, Washington, New York, New York, New Orleans, Louisiana, and San Antonio, Texas

To test the hypothesis that genital colonization with *Ureaplasma urealyticum* would predict adverse pregnancy outcome, 4934 women from five medical centers were evaluated for vaginal colonization with *U. urealyticum* between 23 and 26 weeks' gestation and followed up to delivery. *U. urealyticum* colonization was associated with maternal age, parity, racial-ethnic group, martial status, income, education, smoking, number of sexual partners, and colonization with *Trichomonas vaginalis*, *Mycoplasma hominis*, and bacterial vaginosis. After adjustment for medical and sociodemographic factors in a multivariate analysis, there was no difference in the mean birth weight or proportion of low-birth-weight infants delivered by women who carried *U. urealyticum* and those who did not. *U. urealyticum* colonization at 23 to 26 weeks was not associated with preterm rupture of membranes, preterm labor, or preterm delivery. A positive vaginal culture for *U. urealyticum* in midgestation does not predict those women at risk for preterm labor, preterm delivery, preterm premature rupture of membranes, or delivery of a low-birth-weight infant. (AM J OBSTET GYNECOL 1991;164:728-33.)

Key words: *Ureaplasma urealyticum*, low birth weight, preterm labor, preterm birth, preterm premature rupture of membranes

From the National Institute of Child Health and Human Development,[a] the University of Oklahoma Health Sciences Center,[b] the National Institute of Allergy and Infectious Diseases,[c] the Research Triangle Institute,[d] the University of Washington,[e] Columbia University,[f] the Louisiana State University,[g] and the University of Texas at San Antonio.[h]
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Reprint requests: J. Chris Carey, MD, POB 26901, Room 4SP700, Oklahoma City, OK 73190
**Other members of the Vaginal Infections and Prematurity Study Group are listed at the end of the article.*
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Preterm birth and low birth weight are the leading causes of neonatal mortality and morbidity in the United States. The cause of most preterm births is not known, but an increasing body of evidence suggests that genital infections may contribute to preterm labor and preterm rupture of membranes.[1] It has been suggested that commonly recovered vaginal organisms such as *Ureaplasma urealyticum* may cause chorioamnionitis and preterm birth.[2] Recovery of *U. urealyticum* from the infant, the placenta, or the mother's genital tract has been associated with low birth weight[2-5] and histologic chorioamnionitis.[6, 7] However, the association of *U. urea-*

lyticum and pregnancy outcome has not been consistent, and in other studies *U. urealyticum* was not associated with adverse pregnancy outcome.[8,9] Most of the studies have had small numbers or have not controlled for potentially confounding demographic or obstetric factors, and the role of *U. urealyticum* in pregnancy outcome is controversial.

We began a multicenter study in 1984 (the Vaginal Infections and Prematurity Study) to determine the effect of genital microorganisms on pregnancy outcome. A wide variety of microorganisms were cultured from the maternal genital tract to determine their associations with preterm birth. In this report the relationship of *U. urealyticum* carriage at 23 to 26 weeks' gestation with subsequent pregnancy outcome is examined.

Material and methods

Population. The Vaginal Infections and Prematurity Study Group carried out a multicenter, multiyear study that consisted of observation of the relationships between genital colonization with aerobic and anaerobic bacteria, mycoplasmas, *Chlamydia trachomatis, Trichomonas vaginalis, Candida spp.*, demographic factors, medical and obstetric factors, and pregnancy outcome, and a randomized clinical trial of the effect of erythromycin therapy on pregnancy outcome. Women from five medical centers (Columbia Presbyterian Hospital, New York, the University of Texas Health Science Center in San Antonio, Louisiana State University, New Orleans, the University of Washington, Seattle, and the University of Oklahoma Health Sciences Center, Oklahoma City) were included in this study. Women were excluded from the study for the following reasons: insulin-dependent diabetes mellitus, current use of corticosteroids, chronic renal disease (serum creatinine level > 2.5 mg/dl), current use of digitalis, symptomatic organic heart disease, plans to deliver at a nonstudy hospital, multiple gestation, prior use of tocolytic agents during the current pregnancy, cervical cerclage, hypertension requiring medication, age < 16 years, Rh isoimmunization, or antibiotic use in the preceding 2 weeks. All women gave written informed consent approved by the respective institutional review boards of the participating centers.

Women were interviewed between 23 and 26 completed weeks' gestation. A standardized medical, obstetric, sexual, and social history and cultures of the vagina, cervix, and urine were obtained from all women on admission to the study. All women were followed up to delivery. If a woman was lost to follow-up or delivered at another hospital, an effort was made to determine the date of delivery, sex and birth weight of the infant, and any additional information about the maternal and neonatal course. Women who presented with labor or rupture of membranes before 37 weeks had vaginal and cervical cultures taken during labor, when possible. Women in labor at term during regular weekday working hours also had cultures taken.

The study included a randomized, placebo-controlled, double-masked clinical trial of erythromycin therapy for women who carried *U. urealyticum, C. trachomatis* or β-hemolytic group B streptococci. All women who carried group B streptococci or *C. trachomatis,* as well as a subset of those with *U. urealyticum,* were approached for enrollment into the clinical trial. To reduce problems with interpretation of the role of *U. urealyticum* in adverse pregnancy outcome, we excluded all women with group B streptococci or *C. trachomatis* and those women with *U. urealyticum* who were randomized into the clinical trial from these analyses. The results of the clinical trial will be presented in a separate report. Women who had >100,000 colony-forming units per milliliter isolated from urine cultures or who had *Neisseria gonorrhoeae* isolated from the cervix were treated and followed up in the observational study but were ineligible for the clinical trial. Women were not otherwise treated on the basis of the enrollment cultures.

Gestational age was determined by means of a combination of last menstrual period, early obstetric examination, and ultrasonographic evaluation, where indicated. Preterm labor was defined as spontaneous onset of regular uterine contractions before 37 weeks that led to delivery or the administration of parenteral tocolytic agents. Premature rupture of membranes was defined as spontaneous membrane rupture before the onset of regular uterine contractions, and preterm premature rupture of membranes occurred before 37 completed weeks' gestation. Low birth weight was defined as a birth weight <2500 gm, and very low birth weight was defined as a birth weight <1500 gm.

Microbiology. Specimens for *U. urealyticum* were obtained by washing the vagina with 3 ml of prereduced buffered saline solution and aspirating the wash. Shepard's A7-B agar plate was then inoculated with 0.05 ml of the wash, and the plate was streaked into four quadrants with a sterile swab premoistened with prereduced saline solution. Kenny's MES media[10] were then inoculated with 0.1 ml of the wash. Agar plates were incubated aerobically at 37° C for 3 days and observed for colony morphologic features. The broth was incubated for up to 1 week or until a pH color shift was observed. On the day of color shift the broth was subcultured or frozen at −20° C for later subculture in batch. The subculture was streaked on an agar plate and again placed in broth. Typical colony morphologic features were required from subcultured broth to confirm the presence of *U. urealyticum* when no organisms were found on the original agar plates.

Table I. *U. urealyticum* isolation by demographic variables

Variable	*No.*	*Percent U. urealyticum positive*	*p Value (χ^2)*
Race/ethnicity			<0.001
Black	1413	77.8	
Hispanic	1584	61.2	
White and other	1936	60.9	
Gravidity			<0.001
Primigravid	1229	72.4	
Multigravid	3519	63.6	
Age at enrollment			<0.001
16-17 yr	372	77.7	
18-19 yr	700	74.4	
20-24 yr	1739	67.7	
25-29 yr	1232	63.1	
≥30 yr	886	54.1	
Marital status			<0.001
Married	2549	57.8	
Separated/divorced	742	69.4	
Never married	1638	76.7	
Education			<0.001
<12th grade	2059	69.6	
12th grade	1791	66.4	
>12th grade	1077	57.9	
Income			<0.001
<$5,000	1419	70.9	
$5,000-9,900	1190	68.4	
$10,000-14,900	689	59.4	
≥$15,000	631	52.1	
No. of sexual partners in last year			<0.001
1	4191	63.9	
2	425	76.2	
≥3	225	81.8	
Smoking during pregnancy or previous 3 mo			<0.001
Yes	1823	70.9	
No	3136	63.2	
Drinking in pregnancy or previous 3 mo			NS
Yes	1974	66.2	
No	2985	65.8	
History of marijuana use during pregnancy			<0.01
Yes	315	72.7	
No	4609	65.5	
History of cocaine use in pregnancy			<0.05
Yes	58	79.3	
No	4886	65.8	

Statistical methods. Data were entered onto paper forms and mailed to the Data Coordinating Center where the forms were entered into a mainframe computer. Data forms were edited and returned to the clinical centers for corrections where indicated. Data analysis was done with the Statistical Analysis System.* Continuous outcomes were analyzed with a two-sample *t* test or linear regression methods. Associations between *U. urealyticum* and dichotomous outcomes are summarized with the odds ratio, which is a good estimate of the relative risk in this circumstance. Adjustment for covariates was done by stratified analysis using the χ^2 test of Mantel and Haenszel with no continuity correction and by logistic regression analysis.

**SAS is the registered trademark of SAS Institute, Inc, Cary, NC 27511.*

Results

From Nov. 1, 1984, until June 30, 1987, 8287 women were enrolled in the study (2600 black, 2774 Hispanic, 2675 white, and 238 women of Asian, Native American, or other racial or ethnic groups). Of these 1885 women were randomized into the clinical trial, 1359 women had group B streptococci or *C trachomatis,* and 109 of the remaining women had no evaluation for *U. urealyticum* carriage, leaving 4934 women for the analysis of *U. urealyticum* carriage and pregnancy outcome. Of these 4568 had delivery information available.

U. urealyticum was recovered from 72% of the 8287

women initially enrolled and 65.9% of the 4934 women studied in this analysis, making it the most frequently isolated organism in this study. The organism was recovered from 89% of 1511 women from the Louisiana State University, 77% of 594 women from the University of Washington, 70% of 2504 women from Columbia Presbyterian Hospital, 70% of 1244 women from the University of Texas, and 68% of 2254 women from the Oklahoma University Health Sciences Center. The associations of *U. urealyticum* carriage with behavioral and demographic factors are shown in Table I. *U. urealyticum* was recovered more commonly from black than Hispanic or white women and from primigravid women more commonly than multigravid women. The carriage rate of *U. urealyticum* declined significantly with increasing age (p for trend < 0.001), with increasing income (p for trend < 0.001), and with increasing education (p for trend < 0.001). Married women had significantly lower rates of carriage than either unmarried or previously married women. We isolated *U. urealyticum* significantly more often from women who smoked during or in the 3 months before pregnancy than in nonsmokers but found no difference between those who drank alcohol and those who did not. *U. urealyticum* carriage was strongly associated with increasing numbers of sexual partners in the last year (p for trend < 0.001), as well as with increasing numbers of sexual partners during the pregnancy or in the woman's lifetime (data not shown).

U. urealyticum carriage was significantly associated with recovery of *T. vaginalis* and *N. gonorrhoeae* but not *Candida albicans* (Table II). *U. urealyticum* was also associated with bacteria vaginosis (on the basis of Gram's stain diagnosis), and the organisms often isolated in association with this syndrome (*Gardnerella vaginalis, Bacteroides spp., Mycoplasma hominis,* anaerobic lactobacilli, and peptococci).

Pregnancy outcomes. Seven percent (322/4453) of the infants born to women in this analysis weighed <2500 gm and 1.4% (64/4453) weighed <1500 gm. The mean birth weight of all infants in this analysis was 3282 gm. Eleven percent (511/4520) were delivered before 37 weeks, 10.4% (466/4529) had preterm labor, and 3.1% (141/4529) had preterm premature rupture of membranes.

Women who carried *U. urealyticum* were slightly more likely to be delivered of a low-birth-weight infant than were those who did not (7.9% vs 6.1%, $p = 0.02$, Table III). Women who carried *U. urealyticum* were also more likely to be delivered of a very-low-birth-weight-infant (1.6% vs 0.9%, $p = 0.04$, Table III). To determine if *U. urealyticum* carriage was an independent risk factor for low birth weight, we performed a stepwise multiple logistic regression. After adjusting for ethnicity, smoking, parity, and carriage of *T. vaginalis,* we found that *U. urealyticum* was not significantly associated with low birth weight (Table IV) or very low birth weight. Addition of other potential confounders to the model had no effect.

Table II. Association of *U. urealyticum* with other organisms

Organisms	*Total*	*Percent U. urealyticum positive*	*p Value*
T. vaginalis			
Pos.	511	80.0	<0.01
Neg.	4380	64.2	
M. hominis			
Pos.	1371	85.7	<0.01
Neg.	3562	58.2	
N gonorrhoeae			
Pos.	54	81.5	<0.01
Neg.	4873	65.6	
G. vaginalis			
Pos.	2499	75.8	<0.01
Neg.	2352	54.9	
Bacteroides			
Pos.	739	80.5	<0.01
Neg.	4173	63.2	
Peptococci			
Pos.	281	78.3	<0.01
Neg.	4631	65.1	
Anaerobic lactobacilli			
Pos.	153	73.9	<0.01
Neg.	4759	65.6	
Aerobic lactobacilli			
Pos.	3431	64.5	<0.01
Neg.	1495	69.1	
C. albicans			
Pos.	312	68.3	NS
Neg.	4617	65.7	
Bacterial vaginosis (by Gram's stain)			
Pos.	1573	78.6	<0.01
Neg.	3299	60.1	

Pos., Positive; *neg.*, negative.

Women who carried *U. urealyticum* were delivered of infants with a mean birth weight of 3276 gm (SD, 583 gm) and those who did not had infants with a mean birth weight of 3325 gm (SD, 559 gm; $p < 0.01$). After adjustment for parity, ethnicity, and smoking during pregnancy, the adjusted mean difference attributable to *U. urealyticum* was 22 gm (F 1.37, $p > 0.2$), and after adjustment for those factors plus marital status, income, ethanol use, marijuana use, and isolation of *T. vaginalis, M. hominis,* or *N. gonorrhoeae,* the adjusted mean difference attributable to *U. urealyticum* was 1 gm (F 0.00, $p > 0.95$).

There was no significant difference in the rates of preterm delivery, preterm labor, or preterm premature rupture of membranes between women who had positive cultures for *U. urealyticum* and those who did not (Table III). Stratifying these rates by race or ethnic

Table III. Pregnancy outcomes as related to *U. urealyticum* carriage at 23 to 26 weeks' gestation

Outcome	*U. urealyticum–positive*	*U. urealyticum–negative*	*p Value*
Labor <37 wk	10.4% (306/2940)	10.2% (160/1564)	0.9
Delivery <37 wk	11.4% (342/3001)	11.0% (172/1567)	0.6
Preterm premature rupture of membranes	3.1% (90/2940)	3.3% (51/1564)	0.6
Birth weight <2500 gm	7.9% (237/3012)	6.1% (96/1587)	0.02
Birth weight <1500 gm	1.6% (49/3012)	0.9% (14/1587)	0.04

Table IV. Odds ratios for pregnancy outcomes in *U. urealyticum*–positive women

Outcome	*Unadjusted odds ratio*	*95% Confidence interval*	*Adjusted*	*95% Confidence interval odds ratio*
Birth weight <2500 gm	1.3	1.05-1.7	1.2	0.95-1.6
Birth weight <1500 gm	1.8	0.99-3.1	1.4	0.8-2.6
Delivery before 37 wk	1.1	0.9-1.3	1.0	0.8-1.2
Labor before 37 wk	1.0	0.8-1.2	1.0	0.8-1.2
Preterm premature rupture of membranes before 37 wk	0.9	0.6-1.3	0.9	0.6-1.2

group, site, or other variables did not alter these results. Stepwise multiple logistic regressions examining the relationship of *U. urealyticum* carriage with preterm labor, preterm delivery, and preterm premature rupture of membranes showed that colonization with *U. urealyticum* in midgestation was not a significant predictor of preterm birth, preterm delivery, or preterm premature rupture of membranes after adjustment for potential confounders (Table IV).

We performed similar analyses of *U. urealyticum* carriage and the association between *U. urealyticum* colonization and outcome, including the women who were randomized in the clinical trial. No important differences were noted in the risk factors for *U. urealyticum* isolation or in the association between *U. urealyticum* isolation and pregnancy outcome (data not shown).

Comment

This study agrees with previous studies, which have failed to show an association between genital colonization with *U. urealyticum* and pregnancy outcome.[8, 9, 11, 12] Previous reports linking *U. urealyticum* to pregnancy outcome have shown an association between isolation of the organism from the placenta,[5, 6, 13, 14] the newborn infant,[7, 10] or genital cultures taken at delivery[8] and preterm birth or low birth weight. Preterm labor has been associated with other genital microorganisms such as group B streptococci,[15] *C. trachomatis*,[16] and bacteria[17, 18] and with bacterial vaginosis.[19] It is therefore important to control for the presence of other organisms when associations with pregnancy outcome are studied. We found an association between isolation of *U. urealyticum* and other genital microorganisms and an association between *U. urealyticum* and sociodemographic factors previously reported to be associated with low birth weight.[20] Although we found a weak association between antepartum colonization with *U. urealyticum* and low birth weight on univariate analysis, this association was not significant after we controlled for these sociodemographic factors and other microorganisms. Although we cannot exclude a role for *U. urealyticum* in low birth weight, our analysis indicates that the association between *U. urealyticum* carriage in the lower genital tract and low birth weight is weak and of minimal clinical significance when compared with these other factors.

The analyses presented here exclude women who were in the randomized clinical trial of erythromycin therapy, because the trial results will be published in a separate report. Our results could have been influenced by a selection bias if women in the trial with *U. urealyticum* had a different risk for adverse outcome than those who were not in the trial. Consequently we performed similar analyses that included women in the trial, and our conclusions were the same as those we have reported. It is possible that women with *U. urealyticum* and either group B streptococci or *C. trachomatis* were at higher risk. We chose to exclude these women to clearly see an effect of *U. urealyticum* on pregnancy outcome.

The results of this study indicate that antepartum genital cultures for *U. urealyticum* are not of value in predicting pregnancy outcome. Women who carried *U. urealyticum* were no more likely to have preterm premature rupture of membranes, preterm labor, or preterm delivery than those who did not. After adjustment for sociodemographic factors, *U. urealyticum* isolation was not significantly associated with low birth weight.

Routine antepartum cultures for *U. urealyticum* are not justified to predict women at risk for adverse pregnancy outcome.

Other members of the Vaginal Infections and Prematurity Study Group are as follows: Sumner J. Yaffe, MD, Charlotte S. Catz, MD, George G. Rhoads, MD, MPH (now at the University of Medicine and Dentistry of New Jersey), Donald McNellis, MD, Mark A. Klebanoff, MD, MPH, Howard J. Hoffman, MA, and Heinz W. Berendes, MD, MHS, from the National Institute of Child Health and Human Development; Robert Edelman, MD (now at the University of Maryland), Richard A. Kaslow, MD, MPH, Mary Frances Cotch, PhD, and George F. Reed, PhD, from the National Institute of Allergy and Infectious Disease; Arlene Meier, RN, from the University of Oklahoma; Luann Wenthold, RNC, from the Louisiana State University; and W. Kenneth Poole, PhD, and Betty K. Hastings from the Research Triangle Institute.

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A randomized placebo-controlled trial of erythromycin for the treatment of *Ureaplasma urealyticum* to prevent premature delivery

David A. Eschenbach, MD,[a] Robert P. Nugent, PhD,[b] A. Vijaya Rao, PhD,[d] Mary Frances Cotch, PhD,[c] Ronald S. Gibbs, MD,[e] Kathleen A. Lipscomb, RN,[e] David H. Martin, MD,[f] Joseph G. Pastorek, MD,[f] Philip J. Rettig, MD,[g] J. Christopher Carey, MD,[g] Joan A. Regan, MD,[h] Kim L. Geromanos, RN,[h] Molly L.F. Lee, RNC,[a] W. Kenneth Poole, PhD,[d] Robert Edelman, MD,[c] and the Vaginal Infections and Prematurity Study Group*

Seattle, Washington, Bethesda, Maryland, Research Triangle Park, North Carolina, San Antonio, Texas, New Orleans, Louisiana, Oklahoma City, Oklahoma, and New York, New York

Ureaplasma urealyticum has been associated with low birth weight and histologic chorioamnionitis and it is a frequent isolate from the chorioamnion of patients who are delivered prematurely. In prior clinical trials using antibiotics active against *U. urealyticum*, antibiotic treatment was associated with reduced prematurity and increased mean birth weight. In this multicenter, randomized, double-blind clinical trial, pregnant women with *U. urealyticum* were treated with 333 mg of erythromycin base or placebo three times daily, starting between 26 and 30 weeks' gestation and continuing through 35 completed weeks of pregnancy. Women with urinary tract infection or *Neisseria gonorrhoeae* infection were excluded from the trial, and women with *Chlamydia trachomatis* or group B streptococci were excluded from these analyses. Erythromycin did not eliminate *U. urealyticum* from the lower genital tract. There were no significant differences between erythromycin- and placebo-treated women in infant birth weight or gestational age at delivery, in frequency of premature rupture of membranes, or in neonatal outcome. (Am J Obstet Gynecol 1991;164:734-42.)

Key words: *Ureaplasma urealyticum*, erythromycin, low birth weight, preterm birth, preterm premature rupture of membranes, clinical trial

An association between low birth weight and *Ureaplasma urealyticum* was first noted in a report of lower mean birth weight among infants with *U. urealyticum* in the nose and throat when compared with infants without the organism.[1] In these early studies an association was also found between birth weight and *U. urealyticum* recovery from a maternal genital culture[2] and between histologic chorioamnionitis and the recovery of the organism from the mother or infant.[3] More recently, histologic chorioamnionitis and poor pregnancy outcome also have been associated with recovery of *U. urealyticum* between the chorion and amnion.[4-6]

A role of *U. urealyticum* in prematurity has also been implied from two antibiotic trials. In a study conducted from 1963 to 1965, nonbacteriuric pregnant women receiving tetracycline were delivered of fewer premature live-born infants than placebo-treated women.[7] Subsequently erythromycin or placebo was administered to pregnant women with either *U. urealyticum* or *Mycoplasma hominis*.[8] Among women who were treated in the third trimester and who were considered to have successfully completed 6 weeks of therapy, patients who received erythromycin were delivered of infants with significantly higher birth weight as compared with those who received placebo.

However, other data suggesting that *U. urealyticum* caused prematurity, premature rupture of the membranes, or low birth weight have not been as convincing. In a recent large prospective study, *U. urealyticum* was

From the University of Washington,[a] the National Institute of Child Health and Human Development[b] and the National Institute of Allergy and Infectious Diseases,[c] National Institutes of Health, Research Triangle Institute,[d] the University of Texas Health Science Center,[e] the Louisiana State University,[f] the University of Oklahoma,[g] and Columbia University.[h]

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Reprint requests: Robert Nugent, National Institutes of Health, EPS 750, Bethesda, MD 20892.
**Other members of the Vaginal Infections and Prematurity Study Group are listed at the end of the article.*
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not related to prematurity.[9] The recovery of *U. urealyticum* from amniotic fluid of women in premature labor was not associated with premature delivery, whereas recovery of bacteria from amniotic fluid was highly associated with premature delivery within a day.[10]

This randomized, double-blind, controlled trial was designed to assess the effectiveness of erythromycin in preventing prematurity among women with *U. urealyticum*. Pregnant women had cultures taken for *U. urealyticum* and other known genital pathogens, except for viruses.

Material and methods

Study design. As part of a multicenter Vaginal Infection and Prematurity Study, pregnant women were enrolled in five antepartum clinics (Columbia Univeristy, New York; Louisiana State University, New Orleans; University of Oklahoma, Oklahoma City; University of Texas Health Science Center, San Antonio; and University of Washington, Seattle). Representatives from each of the centers and from Research Triangle Institute and the National Institutes of Health agreed to a uniform protocol, including questionnaire and laboratory methods. Women seeking prenatal care between the twenty-third and twenty-sixth completed week of pregnancy were screened for eligibility to enter the observational phase of the study. Women were eligible if they were ≥16 years old, were free of medical conditions (hypertension, severe renal or cardiac disease requiring medication, diabetes requiring insulin) and obstetric conditions (erythroblastosis, multiple gestation, cervical cerclage) related to premature delivery, and were not taking selected medications (antibiotics within the previous 2 weeks or current tocolytic or steroid use). Patients who agreed to participate signed a consent form approved by the human subjects protection committee at each institution. Research personnel obtained demographic, obstetric, sexual, contraceptive, and drug use history on standardized coded forms; performed a standardized physical and pelvic examination; and collected urinary-genital cultures in a standard manner.

Patients whose screening cultures contained *Neisseria gonorrhoeae* or $\geq 10^5$ microorganisms per milliliter in a urine culture were treated and were ineligible for the randomized clinical trial. Other criteria for exclusion from the trial included allergy to erythromycin, use of antibiotics since the screening examination and cultures, current use of theophylline (because it reacts with erythromycin), and a history of jaundice or liver disease within the past 3 years. Women carrying *U. urealyticum*, group B streptococci, and *Chlamydia trachomatis* were considered for randomization into the trial. However, patients with *C. trachomatis* or group B streptococci (with or without *U. urealyticum*) have been removed from these analyses. Results of the trial for patients with group B streptococci and *C. trachomatis* will be reported separately.

Patients who had *U. urealyticum*, group B streptococci, or *C. trachomatis* recovered at the screening visit were invited by telephone or in person to participate. Women who agreed to participate in the trial signed a second consent form and entered a 1-week placebo run-in that has been described in further detail elsewhere.[11] Patients who took less than two thirds of the pills or did not return to the clinic were dropped from consideration for the randomized trial.

Women who successfully completed the placebo run-in, still agreed to participate, had none of the exclusion criteria, and were at <30 weeks' gestation were randomized into the double-blind, placebo-controlled trial. Numbers corresponding to packets of either erythromycin or placebo were individually assigned to patients by Research Triangle Institute, using a balanced randomization scheme, at the time of a telephone call for each enrollee. The randomization was done by computer according to a permuted-block procedure with random block sizes (randomly selected between two, four, and six). The randomization was done within strata defined by clinical centers and the seven combinations of the three microorganisms under study. Patients received either erythromycin base, 333 mg, or identical-appearing placebo tablets to be taken three times daily from blister packs containing 21 pills. The erythromycin and placebo were kindly supplied by The Upjohn Company, Kalamazoo, Mich.* Patients were given the telephone number of study personnel and encouraged to report any side effects or difficulties in compliance. The women were elevated at each regular antenatal visit, at which time compliance (by pill count) and side effects were discussed with the research nurse using a standardized questionnaire; used blister packs were collected; and additional medication was supplied. Women were instructed to take the study medication for 10 weeks or until the end of the thirty-fifth week of pregnancy, whichever came first.

Repeat genital cultures were obtained between 31 and 34 weeks' gestation from the first 100 women enrolled into the clinical trial at each center. In addition, a randomly selected sample of 12% of patients originally enrolled in the observational study were asked to return for a repeat of all genital cultures, including

*The use of this medication does not represent an endorsement by the National Institutes of Health or the U.S. Public Health Service.

U. urealyticum (6% at 31 to 33 weeks' gestation and 6% at 34 to 36 weeks' gestation). Genital cultures also were obtained from women admitted for preterm pregnancy complications at <37 weeks' gestation (premature labor, rupture of membranes, etc.) and from patients admitted to the hospital in term labor during daytime hours of weekdays. Patients admitted to the hospital for pregnancy complications or in labor had pertinent pregnancy, labor, and delivery information collected on standardized forms.

Genital examination and collection of urogenital specimens. A clean, unlubricated speculum was placed into the vagina; the appearance of the vaginal discharge and pH were noted. Sterile cotton swabs were used to obtain vaginal material from the posterior vaginal fornix for the culture of *Trichomonas vaginalis* and group B streptococci and for vaginal Gram's stain. Prereduced sterile phosphate-buffered saline solution (3 ml) was placed into the vagina, and vaginal discharge was mixed with the solution by swabbing discharge from the lateral vaginal walls into the solution. The material was then removed with a needle and syringe and injected into an air-free tube for transport to the microbiology laboratory. All cultures were placed on media within 6 hours of collection.

Vaginal discharge was wiped from the cervical portio, and the appearance of endocervical mucus and the cervix was noted. Sterile cotton swabs were used to obtain endocervical material for Gram's stain and *N. gonorrhoeae* and group B streptococcal culture, and a sterile Dacron swab was used to obtain a *C. trachomatis* culture. Midstream urine cultures were collected after women had washed the perineal area with a cleansing solution placed on sterile gauze. Urine was plated onto MacConkey's media with a standard calibrated loop.

Culture methods. Vaginal wash (0.05 ml) was placed on solid mycoplasma media[12] and into separate liquid media for the recovery of *U. urealyticum* and *M. hominis*.[13] Soy extract was kindly supplied by George Kenny, University of Washington from lots that supported adequate mycoplasma growth. Both of the genital mycoplasmas were identified by typical morphologic features on solid media. Liquid media were examined daily for 7 days, and a color change indicating growth was confirmed by identifying typical colonies on subculture onto solid media.

Definitions. Gestational age was estimated from the last menstrual period, subject to correction from pregnancy test, first pelvic examination, fetal heart tone, and available ultrasonographic information. Premature labor was defined as spontaneous onset of regular uterine contractions before 37 weeks that led to delivery or the administration of parenteral tocolytic agents. Premature rupture of membranes was defined as rupture of membranes at any time before the onset of regular uterine contractions.

Statistical analysis. The coded questionnaires on history, physical examination, and laboratory results were sent to Research Triangle Institute, where the data were keyed, verified, and edited. The data were analyzed by means of analysis of variance for continuous variables and contingency table methods for categoric variables. Also χ^2 tests for association were used to test for differences in the distribution of categoric variables between treatment groups. When treatment group comparisons were adjusted for a single categoric factor, a Mantel-Haenszel test was done. However, the p values given in the tables are for tests of the differences between the two treatment groups for patients in specific strata; they are not based on Mantel-Haenszel statistics. Further analysis of the data was done with a logistic regression model, which included all the stratification factors used in Table IV except compliance. The efficacy of the clinical trial was periodically monitored by conditional power techniques. These procedures permit the estimation of the probability of observing a statistically significant difference at the end of the trial, given the information accumulated at some time point before the trial's termination date. The statistical computations were done with SAS procedures.*

Results

Between Nov. 1, 1984, and June 30, 1987, 8287 pregnant women were enrolled into the observational phase. *U. urealyticum* was recovered from 5998 women (72%), including 4302 women who had *U. urealyticum* recovered but did not have group B streptococci, *C. trachomatis*, *N. gonorrhoeae*, or a urinary tract infection. Of these 4302 patients with *U. urealyticum* alone, 245 women were ineligible for the clinical trial and 2113 women were not, or could not be, contacted for enrollment into the clinical trial. Of the remaining 1944 women who were both eligible and contacted for the clinical trial, 69 women (4%) decided against enrollment, 436 (22%) did not comply with the placebo run-in, and 258 (13%) were not enrolled into the run-in, mainly because of appointment failures. The remaining 1181 (61%) of 1944 women were randomized into the double-blind trial; 605 women received erythromycin and 576 women received placebo (Table I). Repeated attempts were made to obtain data on patients who were delivered elsewhere or who were lost to follow-up; complete

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Table I. Characteristics of *U. urealyticum*–positive patients who were randomized to receive either erythromycin or placebo

	Erythromycin (N = 605)	*Placebo (N = 576)*
Age (yr, mean ± SD)	23.9 ± 5.3	23.6 ± 5.6
Gravidity (mean ± SD)	2.6 ± 1.5	2.6 ± 1.7
Parity (mean ± SD)	1.0 ± 1.2	1.0 ± 1.2
Ethnicity		
White	199 (33%)	193 (33%)
Black	149 (25%)	137 (24%)
Hispanic	245 (40%)	239 (42%)
Other	14 (2%)	7 (1%)
Marital status		
Married	280 (46%)	301 (52%)
Separated/divorced/widowed	111 (18%)	72 (13%)
Never married	214 (36%)	203 (35%)
Education (yr, mean ± SD)	11.1 ± 2.1	11.3 ± 2.1
Household income:		
<$5,000	176 (29%)	174 (30%)
$5,000-$9,999	173 (29%)	148 (26%)
$10,000-$14,999	106 (17%)	86 (15%)
>$15,000	37 (6%)	46 (8%)
Refused/unknown	113 (19%)	122 (21%)
Gestational age when screened (wk, mean ± SD)	24.3 ± 1.1	24.3 ± 1.1
Gestational age when treated (wk, mean ± SD)	29.0 ± 1.1	29.0 ± 1.4

Table II. Recovery of vaginal *U. urealyticum* after 4 weeks of erythromycin or placebo

	U. urealyticum recovered/No. cultured	
Center	*Erythromycin*	*Placebo*
University of Texas	28/30 (93%)	35/40 (88%)
Louisiana State University	23/26 (88%)	21/25 (84%)
University of Washington	21/23 (91%)	32/35 (91%)
Columbia University	28/36 (78%)	23/28 (82%)
University of Oklahoma	26/44 (59%)	37/49 (76%)
TOTAL	126/159 (79%)	148/177 (84%)

$p > 0.05$, Effect of therapy on eradication adjusted for center.

data were obtained for 95% of patients enrolled into the trial.

Enrollment in the *U. urealyticum* portion of the clinical trial was terminated in July 1987 on the recommendation of the Data and Safety Monitoring Committee. The committee's decision was based on conditional power calculations, which revealed a very low probability of observing a statistically significant difference in outcomes between the treatment groups in patients with *U. urealyticum* if the trial continued to its planned termination date of March 1989. Also, there was a trend toward increased gastrointestinal side effects in the erythromycin group.

The two randomized groups were compared with respect to several demographic, behavioral, and obstetric characteristics, as shown in Table I. There were no major differences in mean age or demographic, behavioral, or obstetric characteristics between women assigned to erythromycin and those assigned to placebo. The following factors also were not found to differ in patients who received erythromycin and placebo: frequency of smoking, smoking before or during pregnancy; alcoholic drinks in first or last 3 months of pregnancy or before pregnancy; living arrangement; highest year of education; medical payment; working during pregnancy; number of sexual partners during pregnancy, in last year, or during lifetime; frequency of intercourse; prior genital infection or prior kidney infection; cone biopsy; difficulty becoming pregnant; planned pregnancy; hospitalization during pregnancy; mean height or mean weight; general health; illicit drug use; prior spontaneous or induced abortions, stillbirth,

Table III. Birth outcome among women randomly assigned erythromycin or placebo

	Erythromycin	*Placebo*	*p Value*
Birth weight (gm, mean ± SD)	3302 ± 557	3326 ± 558	NS
Birth weight at delivery			
<2000 gm	13/581 (2%)	11/557 (2%)	NS
2000-2499 gm	31/581 (5%)	22/557 (4%)	NS
≥2500 gm	537/581 (92%)	524/557 (94%)	NS
Gestational age at delivery (obstetric estimate)			
≤32 wk	4/583 (0.6%)	7/546 (1.2%)	NS
33-36 wk	47/583 (8%)	40/546 (7%)	NS
≥37 wk	532/583 (91%)	499/546 (91%)	NS
Gestational age at premature rupture of membranes			
≤32 wk	3/578 (0.5%)	3/544 (0.5%)	NS
33-36 wk	13/578 (2%)	9/544 (2%)	NS
≥37 wk	70/578 (12%)	49/544 (9%)	NS
Labor <37 wk	46/578 (8%)	46/544 (8%)	NS
1 min Apgar score			
Mean ± SD	7.7 ± 1.4	7.7 ± 1.2	NS
≤5	40/551 (7%)	36/530 (7%)	NS
5 min Apgar score			
Mean ± SD	8.8 ± 0.6	8.8 ± 0.7	NS
≤5	4/551 (0.7%)	3/530 (0.6%)	NS
Stillbirth	3/590 (0.5%)	3/561 (0.5%)	NS
Neonatal death	1/590 (0.2%)	0/561 (0.0%)	NS

ectopic pregnancy, or low-birth-weight infants; and color, consistency, or amount of vaginal discharge or color of cervical discharge.

At initial screening examination, conducted between 23 and 26 completed weeks of pregnancy, *U. urealyticum* was recovered from 89% of 1511 patients at Louisiana State University, 77% of 594 patients at University of Washington, 70% of 2504 patients at Columbia University, 70% of 1244 patients at University of Texas, and 68% of 2254 patients at University of Oklahoma. *U. urealyticum* isolation after approximately 4 weeks of either erythromycin or placebo treatment (at 31 to 36 weeks' gestation) is listed for each of the five centers in Table II. The differences in the recovery of *U. urealyticum* between the erythromycin and placebo patients were not statistically significant at the $p = 0.05$ level, with or without adjustment for center. Among patients who were delivered before 37 weeks' gestation, *U. urealyticum* was recovered from 18 (86%) of 21 patients who received erythromycin and 15 (94%) of 16 patients who received placebo. Among those who were delivered at or beyond 37 weeks of pregnancy, *U. urealyticum* was recovered from 102 (76%) of 135 patients who received erythromycin and 96 (78%) of 123 patients who received placebo. The recovery of *U. urealyticum* at delivery did not differ significantly between the erythromycin and placebo groups or between women with or without rupture of membranes before culture. The semiquantitative concentration of *U. urealyticum* in the lower genital tract also was not appreciably changed after either erythromycin or placebo therapy.*

A comparison of selected outcome variables among patients who received erythromycin and placebo are shown in Table III. Of all patients entered into the double-blind clinical trial, the mean birth weight of infants was similar in the erythromycin and placebo groups. The distribution of birth weight by standard weight groups and the obstetric estimate of gestational age at delivery were also similar between the erythromycin and placebo groups. There were similar proportions of patients in the two groups with premature rupture of the membranes, premature labor before 37 weeks' gestation, Apgar scores ≤5, stillbirths, and neonatal deaths.

The frequencies of adverse outcomes were analyzed, with separate adjustment for each of the variables included in Table IV (except for compliance) by means of the Mantel-Haenszel procedure and simultaneously with a logistic regression model. In none of these analyses was the difference between the placebo group and erythromycin group statistically significant at the $p = 0.05$ level.

Subset analyses were undertaken to assess whether a particular subgroup of individuals could have received benefit from erythromycin. The effects of erythromycin versus placebo on birth weight <2500 gm was

*Data available on request from the authors.

Table IV. Effect of erythromycin and placebo therapy on birth weight <2500 gm among pregnancies stratified for selected variables

	Proportion with birth weight <2500 gm/total		
	Erythromycin	*Placebo*	*p Value*
Overall	44/581 (8%)	33/557 (6%)	NS
Center			
Columbia University	13/143 (9.1%)	9/134 (6.7%)	NS
University of Washington	2/36 (5.6%)	3/44 (6.8%)	NS
University of Oklahoma	10/182 (5.5%)	8/161 (5.0%)	NS
University of Texas	11/154 (7.1%)	9/163 (5.5%)	NS
Louisiana State University	8/66 (12.1%)	4/55 (7.3%)	NS
Maternal age			
≤18 yr	5/50 (10%)	3/48 (6%)	NS
18-19 yr	10/96 (10%)	10/95 (11%)	NS
20-24 yr	8/204 (4%)	11/225 (5%)	NS
≥25 yr	21/230 (9%)	9/187 (5%)	0.08
Education			
≤8 yr	8/56 (14%)	2/40 (5%)	NS
9-11 yr	16/228 (7%)	13/209 (6%)	NS
12-16 yr	20/292 (7%)	17/303 (6%)	NS
≥17 yr	0/4	1/4 (25%)	NS
Sex partner(s) last year			
1	35/482 (7%)	29/472 (6%)	NS
>1	9/90 (10%)	4/78 (5%)	NS
Female infant*	24/273 (9%)	15/303 (5%)	0.07
Male infant	20/308 (6%)	18/253 (7%)	NS
Other antibiotic used before enrollment			
Yes	14/152 (9%)	8/104 (8%)	NS
No	30/429 (7%)	25/453 (6%)	NS
Compliance			
Took <⅔ of expected pills	13/138 (9%)	5/95 (5%)	NS
Took ≥⅔ of expected pills	26/417 (6%)	20/434 (6%)	NS
Prior pregnancy outcome†			
Abnormal	12/146 (8%)	10/143 (7%)	NS
Normal	18/282 (6%)	8/238 (3%)	NS
No prior	14/152 (9%)	15/174 (9%)	NS

Other variables tested and not found to be associated with birth weight in the clinical trial include: ethnicity, smoking, alcohol use, marijuana or cocaine use, sexual acitivity or bleeding during pregnancy, and bacterial vaginosis, *T. vaginalis*, *Lactobacillus*, *M. hominis*, or any *Bacteroides spp.* at the first enrollment visit.

*Variables that were signficantly different between the randomized groups. Other variables that differed significantly between the randomized groups but among whom erythromycin therapy did not influence birth weight included type of usual health care and number of prior live-born infants.

†Miscarriage, stillbirth, or infant birth <2500 gm with last pregnancy.

compared for those women considered at high risk for prematurity and for those women with the four characteristics that differed significantly between the erythromycin and placebo groups in this study (Table IV). There were no statistically significant or clinically significant differences between the erythromycin (8%) and placebo (6%) groups in the proportion of infants with birth weight <2500 gm, among patients in any of the subgroups presented in detail in the table, or in any of the groups listed at the bottom of Table IV.

The mean birth weight was significantly increased for erythromycin compared with placebo only for the group with a clinical amniotic fluid infection. The mean birth weight for those with a clinical amniotic fluid infection was 3700 gm for the erythromycin group and 3235 gm for the placebo group ($p = 0.005$).

The effect of erythromycin versus placebo was also compared for delivery before 37 weeks and for premature rupture of the membranes, among the variables listed in Table IV (data not shown). There was no in-

Table V. Comparisons of the rates of compliance, withdrawal, and maternal side effects between the erythromycin-treated group and the placebo-treated group

	Erythromycin (N = 605)	*Placebo (N = 576)*	*p Value*
No. withdrew	82 (14%)	53 (9%)	0.02
Patient compliance	*n = 577*	*n = 543*	
Mean days on ≥2 pills	36.5	38.1	0.03
Mean days on 3 pills	30.7	32.6	0.05
Mean No. of days on pills	38.2	39.7	0.06
Maternal side effects	*n = 586*	*n = 548*	
Appetite loss	91 (16%)	79 (14%)	NS
Nausea	194 (33%)	142 (26%)	0.01
Vomiting	102 (17%)	76 (14%)	NS
Diarrhea	94 (16%)	64 (12%)	0.03
Vaginal or rectal itching	55 (9%)	48 (9%)	NS
Any of above	256 (45%)	218 (40%)	0.06
Maternal bilirubin >1.0	0/179	2/201 (1%)	NS
Serum glutamate pyruvate transaminase more than twice normal	4/178 (2%)	8/196 (4%)	NS

fluence of erythromycin on delivery before 37 weeks' gestation or on premature rupture of membranes within any of these groups.

More women assigned to erythromycin (14%) than to placebo (9%) withdrew from the study ($p = 0.02$, Table V). The mean number of days patients took at least two pills daily or took all three pills daily and the mean number of days pills were taken were slightly higher in the placebo group than in the erythromycin group ($p < 0.05$ for each analysis). Nausea and diarrhea occurred more frequently in patients who received erythromycin than in those receiving placebo ($p < 0.05$ for each analysis). However, most of the side effects were mild and did not appreciably influence continuation in the study.

Comment

In this multicenter, randomized, double-blind trial there was no appreciable effect of erythromycin therapy on any of the pregnancy outcomes considered here, including birth weight, gestational age at delivery, premature rupture of the membranes, neonatal death, or other measures of neonatal outcome. The results of this study differ from the results of two prior trials of antibiotic treatment to prevent prematurity.[7, 8] In the first trial[7] nonbacteriuric women receiving tetracycline gave birth to significantly fewer premature live-born infants (5%) than women who received placebo (15%). The antibiotic was given for 6 weeks, starting before the thirty-second week of pregnancy. The mean gestational age at delivery in both the antibiotic and placebo group was >38 weeks. It was not possible to associate the effect of the antibiotic with any particular pathogen because genital cultures were not obtained. In the second trial[8] women with genital mycoplasmas (>90% had *U. urealyticum*) were treated with erythromycin (the stearate or estolate form) or placebo starting between the twenty-second and thirty-second week of pregnancy with dose (250 mg four times daily) and duration (6 weeks) similar to those of the present study. The 143 gm increase in birth weight ($p < 0.047$ with a one-tailed test) was evident only among compliant women who completed a 6-week regimen of antibiotic starting in the third trimester, but there was no overall reduction of prematurity among women who had received erythromycin in that study.[8]

There is a major difference in the design of this study compared with the two prior studies. In this analysis women with *C. trachomatis*, group B streptococci, *N. gonorrhoeae*, and urinary tract infections have been excluded. All of these infections have been related to prematurity,[7, 9, 14, 15] and it is possible that antibiotic therapy eliminated them, or other tetracycline-sensitive or erythromycin-sensitive organisms, rather than *U. urealyticum*, in the two prior antibiotic studies.[7, 8]

Although we found no effect of treatment, it is still possible that antibiotic therapy is of benefit for the prevention of prematurity, but treatment may need to be started earlier in pregnancy. The proportion of patients with evidence of upper genital tract infection (of amniotic fluid or chorioamnion) is not constant throughout pregnancy. The prevalence of histologic chorioamnionitis[4, 6, 16] and of microorganisms recovered from the chorioamnioin[6] is highest in the second trimester, intermediate in the early third trimester, and lowest at term. Histologic chorioamnionitis has been related to the recovery of organisms, including *U. urealyticum*, from the space between the chorion and amnion.[4-6] The majority of women in the present study were treated starting at or beyond the twenty-ninth week of gestation, and it is possible that treatment earlier in pregnancy would have been more effective in preventing

infection-related prematurity than therapy begun at this time in pregnancy. Since only 1% of women are delivered of neonates weighing ≤1500 gm at birth (approximately 32 weeks' gestation), a very large number of women would have had to be treated with antibiotics to demonstrate a measurable impact on prematurity if the major preventive effect of antibiotics occurs before 32 weeks' gestation.

A subset of women could be uniquely susceptible to the effect of *U. urealyticum* and hence to prematurity. Potentially, these women could include those with particularly pathogenic strains of *U. urealyticum*, with newly acquired infection or without antibody protection against the microorganism. *C. trachomatis* has been associated with premature delivery and premature rupture of membranes among women with immunoglobulin M, presumably as a result of a newly acquired infection, but not among women with immunoglobulin G antibody, presumably representing chronic carriage of the microorganism.[9] There is evidence that women with a four fold rise in antibody titer to *U. urealyticum* may have an increased risk of premature delivery.[17]

U. urealyticum was not eliminated from the lower genital tract of women receiving erythromycin, either in this trial or in the previous trial.[8] Thus the presence of the microorganisim possibly could have still influenced the pregnancy outcome of both the erythromycin and placebo groups. *U. urealyticum* is susceptible in vitro to erythromycin and tetracycline,[18] but neither antibiotic has effectively eliminated *U. urealyticum* from the lower female genital tract, probably because of the effect of the acidic vaginal pH on the antibiotic.[18] However, lower genital tract *U. urealyticum* would be expected to produce its effect on pregnancy only if it ascended into the upper genital tract. Although tissue concentrations of erythromycin are typically low, erythromycin levels would be expected to be sufficient to inhibit the spread of susceptible microorganisms into upper genital tract sites. Other oral antibiotics that effectively inhibit *U. urealyticum* have toxicity in pregnancy.

The absence of an effect of erythromycin on either *U. urealyticum* colonization or pregnancy outcome might also suggest that some patients did not take the medication. Serum erythromycin levels were not obtained to directly address this issue. Evidence against this position occurs for women in this trial who were colonized with group B streptococci or *C. trachomatis*. In a preliminary report to the Data and Safety Monitoring Committee, erythromycin in the same dose and duration effectively eliminated *C. trachomatis* and group B streptococci. Although gastrointestinal intolerance is a major problem with many erythromycin preparations, the preparation used here was well tolerated.

The absence of an antibiotic effect cannot be explained by a chance failure of the scheme to randomly assign women to erythromycin and placebo groups with different risks for prematurity. The two randomized groups were comparable for characteristics considered to be potential risk factors for prematurity, as well as for numerous other factors noted. There was little chance that the results could be explained by a disproportionate number of patients with other infections previously associated with prematurity. These infections were either excluded from the study (urinary tract infection, *N. gonorrhoeae*), excluded from the analysis (*C. trachomatis*, group B streptococci), or present at similar proportions in the erythromycin and placebo groups (bacterial vaginosis,[19] *T. vaginalis*[20]). The absence of an effect could not be explained by brevity of the antibiotic course, noncompliance, or loss to follow-up, since the mean duration of therapy was 36 days, >80% of the patients took an average of more than two pills per day, and fewer than 15% of randomized patients withdrew from the trial.

We conclude that erythromycin therapy is not justified for the treatment of *U. urealyticum* in the lower genital tract to prevent prematurity.

Other members of the Vaginal Infections and Prematurity Study group are as follow: Sumner J. Yaffe, MD, Charlotte S. Catz, MD, George G. Rhoads, MD, MPH (now at the University of Medicine and Dentistry of New Jersey), Donald McNellis, MD, Mark A Klebanoff, MD, MPH, Howard J. Hoffman, MA, and Heinz W. Berendes, MD, MHS, from the National Institute of Child Health and Human Development; Richard A. Kaslow, MD, MPH, William C. Blackwelder, PhD, and George F. Reed, PhD, from the National Institute of Allergy and Infectious Diseases; Arlene Meier, RN, from the University of Oklahoma; Luann Wenthold, RNC, from the Louisiana State University; and Betty K. Hastings, from the Research Triangle Institute.

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Evaluation of an aneurysm of the vein of Galen in utero by pulsed and color Doppler ultrasonography

Junji Ishimatsu, MD, Osamu Yoshimura, MD, Masahiro Tetsuou, MD, and Teiji Hamada, MD

Fukuoka-Ken, Japan

Pulsed and color Doppler ultrasonographic diagnosis of an anuerysm in one of Galen's veins was achieved prenatally on the basis of the findings of turbulence blood flow in the cerebral cystic lesion and low vascular resistance in the internal carotid artery, the posterior communicating artery, and the posterior cerebral artery. (AM J OBSTET GYNECOL 1991;164:743-4.)

Key words: Prenatal diagnosis, aneurysm of the vein of Galen, pulsed and color Doppler ultrasound, fetal hemodynamics

Described in this article is one case of aneurysm of a Galen's vein in utero, and to our knowledge this is the first report that demonstrates a complete mapping of the fetal intracranial circulation with this phenomenon in conjunction with cardiovascular hemodynamics as evaluated by pulsed and color Doppler ultrasonography.

Case report

A 29-year-old woman, gravida 1, para 1, was referred to Kurume University Hospital at 37 weeks and 1 day of gestation for evaluation of a round intracranial lesion. Color Doppler ultrasonography (Aloka SSD-680, Aloka Co. Ltd., Tokyo) with 3.5 MHz transducer revealed the presence of well-defined cerebral cystic space that measured 29 × 30 × 37 mm and was associated with blood flow within the lesion (Fig. 1) and within the dilated posterior cerebral artery and the straight sinus (7 mm). The blood continued to flow into the cystic lesion through both of the feeding posterior cerebral arteries and to flow into the straight sinus through the cystic lesion. The cystic structure was contiguous to the left lateral ventricle, just superior to the thalamus. The blood flow velocity waveforms of the vessels in the fetal cranium, the abdominal aorta, and the umbilical artery were measured by pulsed Doppler ultrasonography. The resistance index (i.e., peak systolic velocity minus end-diastolic velocity divided by peak systolic velocity) values were significantly elevated in the umbilical artery (0.807), the abdominal aorta (0.962), the anterior cerebral artery (0.850), and the middle cerebral artery (0.886). In contrast, the values were relatively low in the internal carotid artery (0.625) and the posterior communicating artery (0.621), and values were extremely reduced in the posterior cerebral artery (0.356) (Fig. 2). The peak systolic blood flow velocity at the entrance of the cystic lesion was markedly increased (>220 cm/sec) and the resistance index value was 0.318. There was no difference of resistance index value in arteries of bilateral hemispheres. Marked turbulence flow was seen within the cystic lesion and the straight sinus. No structual abnormalities of the fetal heart were seen. The total cardiac dimension of 46 mm exceeded the normal range. Fractional shortenings of the right ventricle (23%) and the left ventricle (33%), ratios between the maximal velocity of early passive ventricular filling and the maximal velocity of active ventricular filling during atrial contraction over the tricuspid valve (0.75) and the mitral valve (0.78), the mean velocities over the tricuspid valve (37 cm/sec) and the mitral valve (36 cm/sec), and the maximum blood flow velocities in the ascending aorta (110 cm/sec) and the pulmonary artery (91 cm/sec) were within the normal range. Retrograde diastolic flow was observed in the aortic arch. Mitral and tricuspid regurgitations were not observed. There were no pericardiac or pleural effusions, ascites, or skin edema. The intracranial abnormal blood flows, the retrograde diastolic flow in aortic arch, and the enlargement of the fetal heart suggested the vascular "steal" phenomenon of the aneurysm of the Galen's vein both of the posterior cerebral arteries. A female infant who weighed 3040 gm was delivered by cesarean section at 38 weeks and 4 days of gestation. The embolizations in both of the feeding posterior cerebral arteries via the internal carotid arteries were successfully achieved on the second and eighth days of life. The infant was discharged on thirty-eighth postnatal day and had not manifested either neurologic or cardiac involvement.

Comment

Prenatal diagnosis of this phenomenon has rarely been done. Only a few cases were reported regarding the Doppler ultrasonographic examination of the fetus

From the Department of Obstetrics and Gynecology, Kurume University School of Medicine.
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Reprint requests: Junji Ishimatsu, Office of the Department of Obstetrics and Gynecology, Kurume University School of Medicine. 67 Asahimachi, Kurume-shi, Fukuoka-ken 830, Japan.
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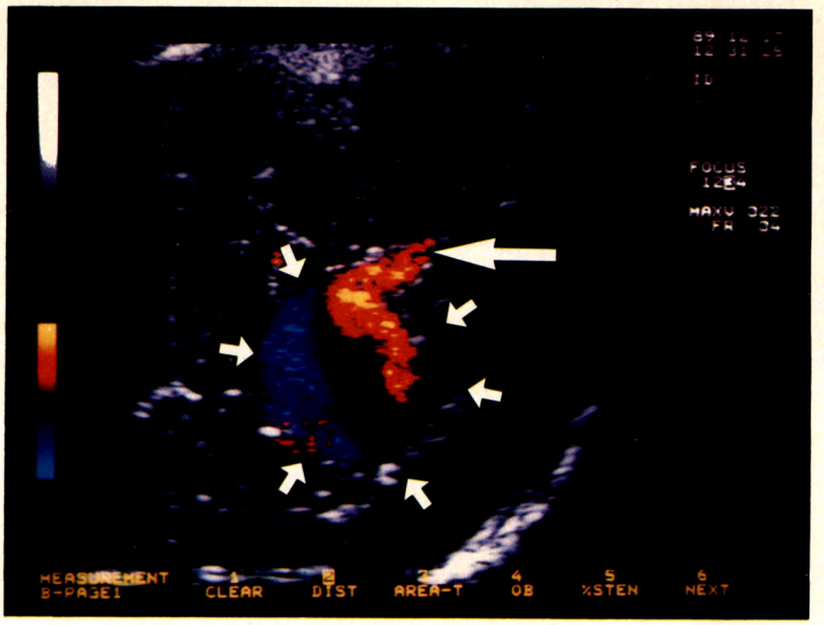


Fig. 1. Color Doppler image (tranverse view) of fetal cranium. Cystic lesion that is slightly superior to thalamus shows aneurysm of Galen's vein *(small arrows)* and dilated straight sinus *(large arrow)*. Red color indicates flow to transducer; blue color indicates flow away from transducer.

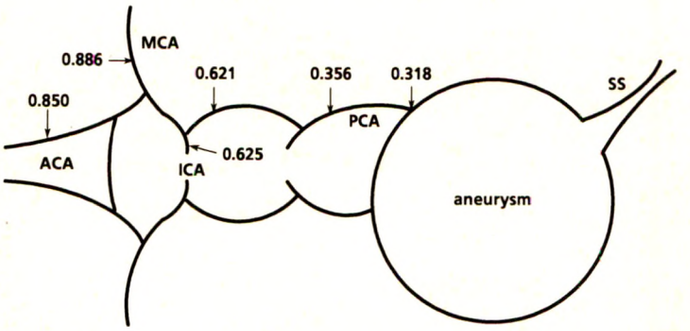


Fig. 2. Resistance index of blood flow velocity waveforms in fetal cerebral arteries. *ACA*, Anterior cerebral artery; *MCA*, middle cerebral artery; *PCA*, posterior cerebral artery; *ICA*, internal carotid artery; *SS*, straight sinus.

with this malformation.[1,2] In the present case, complete mapping of the intracranial vessels by pulsed Doppler ultrasonography permitted us to achieve the precise prenatal diagnosis and the subsequent treatment for favorable prognosis of the infant.

We believe that the pulsed and color Doppler ultrasonography is the most useful noninvasive method for the identification of vascular abnormalities and the evaluation of hemodynamics in the fetus.

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Epidermal growth factor receptor expression in normal ovarian epithelium and ovarian cancer

II. Relationship between receptor expression and response to epidermal growth factor

Gustavo C. Rodriguez, MD,[a] Andrew Berchuck, MD,[a] Regina S. Whitaker, BS,[a] David Schlossman, MD, PhD,[b] Daniel L. Clarke-Pearson, MD,[a] and Robert C. Bast, Jr., MD[b, c]

Durham, North Carolina

Previously we have shown that epidermal growth factor acts as a mitogen for some, but not all, ovarian cancer cells in culture. In this study we examined the effect of epidermal growth factor on proliferation of normal human ovarian epithelial cells in monolayer culture. We found that epidermal growth factor stimulated twofold to fourfold increases in proliferation in epithelial cells from each of five normal ovaries ($p < 0.01$). In addition, Scatchard analysis of binding of epidermal growth factor tagged with iodine 125 indicated the presence of high-affinity receptors in all of the ovarian epithelial cells and ovarian cancer cell lines. The number and affinity of receptors was similar in the normal epithelium and cancer cell lines, and there was no relationship between epidermal growth factor receptor number and responsiveness to epidermal growth factor. We conclude that human ovarian epithelial cells normally express epidermal growth factor receptors and that epidermal growth factor acts as a mitogen for these cells. Although the mitogenic response to epidermal growth factor often is attenuated in ovarian cancer cell lines, loss of responsiveness to epidermal growth factor does not appear to be due to decreased receptor expression.
(Am J Obstet Gynecol 1991;164:745-50.)

Key words: Epidermal growth factor receptor, epidermal growth factor, ovarian cancer

Epidermal growth factor is a 6045 d polypeptide that acts as a mitogen for a wide variety of cells in vitro. The epidermal growth factor receptor is a 170,000 d transmembrane glycoprotein that includes a cysteine-rich, extracellular ligand-binding domain, a short hydrophobic membrane-spanning region, and an intracellular tyrosine kinase domain.[1] In addition to activation of the receptor tyrosine kinase, binding of epidermal growth factor to its receptor stimulates several other biochemical events.[2] Although these "second messengers" are thought to play a role in transmission of the mitogenic signal to the nucleus, our understanding of these events remains incomplete.

The epidermal growth factor receptor is widely expressed in normal human tissues, including both epithelial and mesenchymal cells, and it is thought that epidermal growth factor and its receptor are involved in processes of normal cellular growth and differentiation in vivo. In addition, various abnormalities of the epidermal growth factor receptor and its ligands have been described in human cancers. For example, in squamous cancers it has been noted that epidermal growth factor receptor expression often is increased relative to normal squamous cells.[3] In contrast, it has been reported that only a fraction of breast cancers express epidermal growth factor receptor. In breast cancer, epidermal growth factor receptor expression has correlated inversely with estrogen receptor expression and has been associated with poor survival.[4]

In addition to these observations in human cancers, studies of transformed cells in culture have suggested that malignant cells may escape normal growth regulatory controls by secreting growth factors into their local environment.[5] This phenomenon has been called autocrine growth stimulation. Previously we have explored the role of epidermal growth factor in growth regulation of ovarian cancers. We found that proliferation of some, but not all, ovarian cancer cell lines was increased after treatment with epidermal growth

From the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology,[a] the Department of Medicine,[b] and the Department of Microbiology-Immunology,[c] Duke University, and the Duke Comprehensive Cancer Center.
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Reprint requests: Andrew Berchuck, MD, Duke University Medical Center, P.O. Box 3079, Durham, NC 27710.
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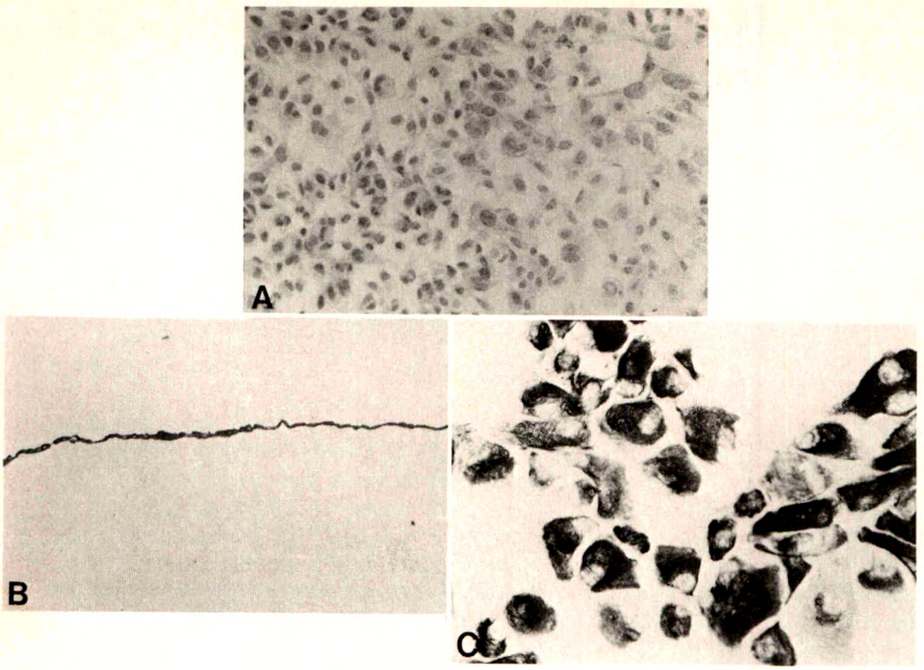


Fig. 1. A, Normal ovarian epithelial cells stained with hematoxylin and eosin. **B,** Normal ovary stained for cytokeratin. **C,** Normal ovarian epithelial cells stained for cytokeratin.

factor.[6] In this study we have examined epidermal growth factor receptor expression in these cancer cell lines to determine whether there is a correlation between responsiveness to epidermal growth factor and receptor number or affinity. In addition, to elucidate whether changes in epidermal growth factor receptor expression occur during malignant transformation, we have examined epidermal growth factor receptor expression and responsiveness to epidermal growth factor of normal human ovarian epithelial cells in culture.

Material and methods

Cell culture. Five epithelial ovarian cancer cell lines (OVCA 420, OVCA 429, OVCA 432, OVCA 433, and DOV 13) previously established in our laboratory were maintained in monolayer culture in tissue culture medium containing modified Eagle's medium (Hazelton Research Products Inc., Lenexa, Kan.) supplemented with 10% heat-inactivated fetal bovine serum (Gibco Laboratories, Grand Island, N.Y.), sodium pyruvate, nonessential amino acids, glutamine, penicillin, and streptomycin. MK (mouse keratinocyte) cells, which are stimulated to proliferate in the presence of epidermal growth factor, were grown in Spinner's modified minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum.

Epithelial cells were obtained from the surface of normal human ovaries that had been removed at operation for treatment of benign gynecologic disease. The ovaries were transferred aseptically from the operating room to the laboratory, where the epithelial cells were isolated by gently scraping the ovarian surface with a scalpel as previously described.[7] The normal ovarian epithelial cells were maintained in monolayer culture in a 1 : 1 mixture of MCDB105 and M199 media (Sigma Chemical Co., St. Louis) supplemented with 10% heat-inactivated fetal bovine serum. The morphologic appearance of these cells in culture was consistent with epithelium (Fig. 1, *A*). The epithelial nature of the cells was verified by immunohistochemical staining with anticytokeratin antibodies AE1/AE3, which bind to ovarian epithelium but not stroma (Fig. 1, *B* and *C*). Purified mouse immunoglobulin G specific for nonhuman tissue (Coulter Immunology, Hialeah, Fla.) was used as a negative control. In addition, electron microscopy was performed to confirm the presence of apical microvilli. Staining with anti-factor-8 antibody was performed to confirm the absence of contaminating endothelial cells.

Thymidine incorporation assay. Ovarian cancer cells were seeded in 96-well plates (5×10^4 cells per well) in tissue culture medium containing 2% fetal bovine serum and different concentrations of murine epi-

dermal growth factor (0, 0.2, 2, or 20 ng/ml) (Sigma). Six replicate wells were incubated with each concentration of epidermal growth factor. After 18 hours, when the cells were confluent, 1 μCi of thymidine tagged with tritium (6.7 Ci/mmol) (New England Nuclear, Wilmington, Del.) was added to each well. Six hours later the culture medium was removed, the cells were washed three times with phosphate-buffered saline solution and solubilized with 2N sodium hydroxide, and cell-associated radioactivity was determined by scintillation counting. MK cells were used in each experiment as positive controls. The normal ovarian epithelial cells were seeded in 96-well plates (5×10^4 cells per well) in MCDB 105–M199 medium containing 2% fetal bovine serum and then allowed to grow to confluence (2 to 3 days). Epidermal growth factor (0, 0.2, 2, or 20 ng/ml) was then added to the cells for 24 hours. During the final 6 hours, [^{3}H]thymidine (1 μCi/well) also was present in each well. Cells were then washed and lysed with 2N sodium hydroxide, and the cell-associated radioactivity was assessed by scintillation counting.

Epidermal growth factor receptor binding. Ovarian cancer cells and normal ovarian epithelial cells were plated in 24-well plates at 1.1 to 1.7 $\times 10^5$ cells per well in their respective culture media and grown to confluence. The monolayers then were washed with Hank's balanced salt solution and incubated at 37° C for 1 hour in medium containing 10% bovine serum albumin and 0.08% sodium azide. Then medium containing 1% bovine serum albumin, 0.4 ng/ml epidermal growth factor tagged with iodine 125 (specific activity 165 mCi/μg) (Amersham Corporation, Arlington Heights, Ill.) and varying concentrations of unlabeled epidermal growth factor (1.6 to 25 ng/ml) were added to the wells. Assays were performed in triplicate. After incubation for 1 hour at 4° C, the cells were washed three times with medium containing 1% bovine serum albumin and then processed for scintillation counting with a γ-counter. Nonspecific binding was determined by incubation of cells with a hundredfold excess of unlabeled epidermal growth factor. The final number of cells per well for each ovarian cancer cell line and normal epithelial culture was determined for each experiment by trypsinization and counting of separate wells. The binding data were analyzed by the Scatchard method with the Enzfitter software program (Elsevier-Biosoft, Cambridge).

Statistics. Student's t test was used to analyze experiments comparing two means. Adjustment for multiple comparisons was made where appropriate.

Results

The effect of epidermal growth factor on [^{3}H]thymidine incorporation of the ovarian cancer cell lines is shown in Fig. 2, *A*. Epidermal growth factor stimulated a threefold increase in [^{3}H]thymidine incorporation in DOV 13 cells ($p < 0.001$). In addition, a 60% to 70% increase was seen in OVCA 433, OVCA 429, and OVCA 420 cells ($p < 0.01$). Proliferation of OVCA 432 cells was not affected by epidermal growth factor. The data shown in Fig. 2, *A*, were found to be reproducible over the course of multiple similar experiments in which the optimal serum concentration was determined. These optimal conditions are what is described in the Material and methods section. The effect of epidermal growth factor on [^{3}H]thymidine incorporation in the normal ovarian epithelial cells is shown in Fig. 2, *B*. In multiple experiments epidermal growth factor stimulated twofold to fourfold increases in [^{3}H]thymidine incorporation in monolayer cultures of normal ovarian epithelial cells from five patients ($p < 0.01$). In each experiment epidermal growth fac-

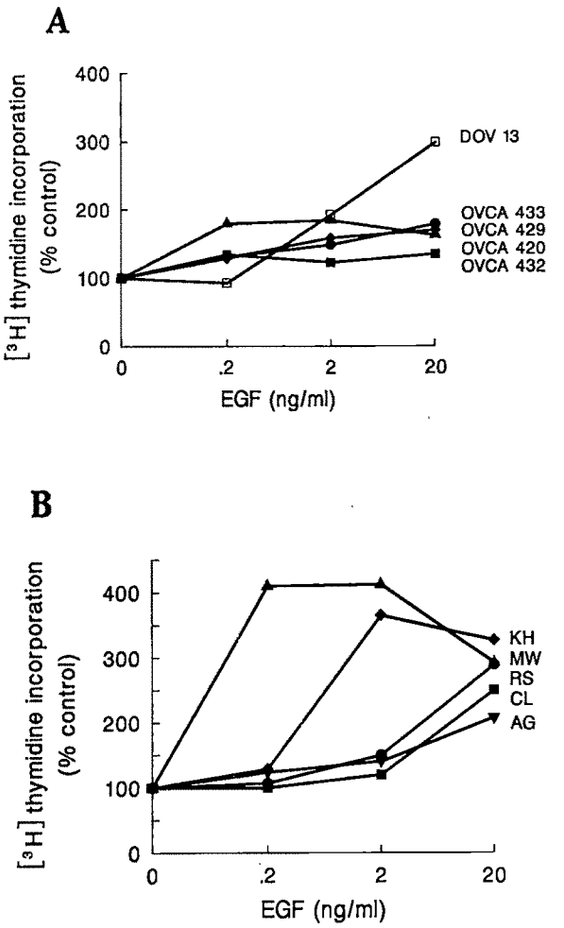


Fig. 2. A, Effect of epidermal growth factor on proliferation of ovarian cancer cell lines (DOV 13, $p < 0.001$; OVCA 433, OVCA 429, and OVCA 420, $p < 0.01$; OVCA 432 = not significant). **B,** Effect of epidermal growth factor on proliferation of normal ovarian epithelial cells ($p < 0.01$).

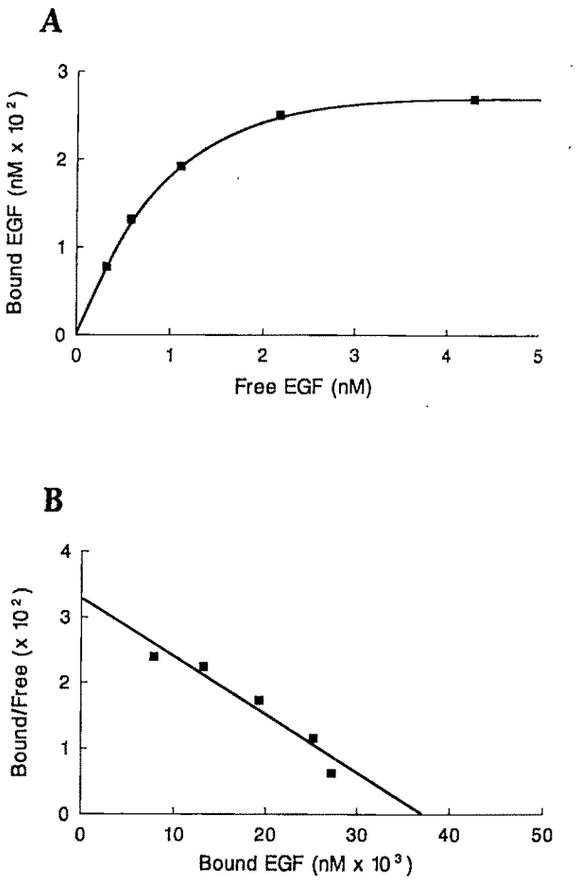


Fig. 3. A, Binding of ^{125}I-epidermal growth factor to DOV 13 ovarian cancer cell line. **B,** Scatchard analysis of ^{125}I-epidermal growth factor binding to DOV 13 cells.

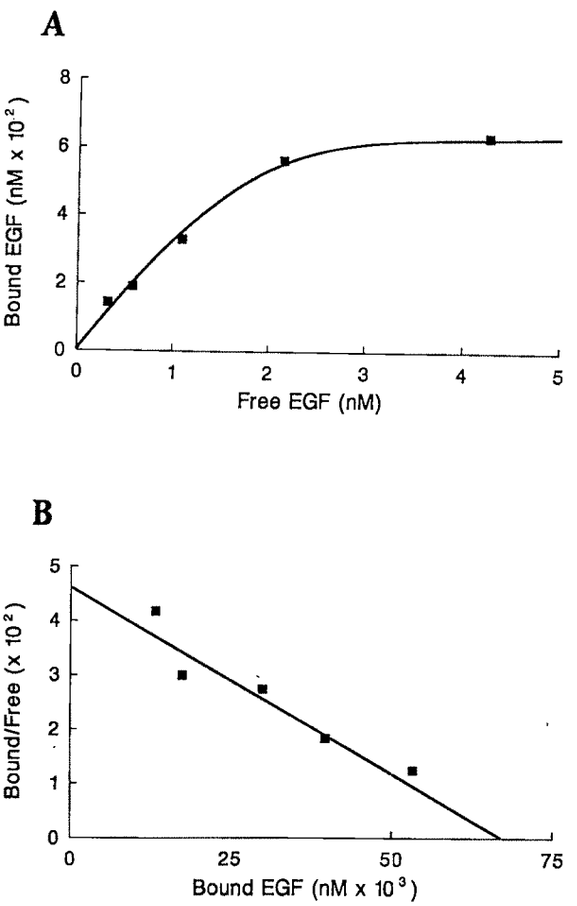


Fig. 4. A, Binding of ^{125}I-epidermal growth factor to normal ovarian epithelial cells. **B,** Scatchard analysis of ^{125}I-epidermal growth factor binding to normal ovarian epithelial cells.

tor markedly stimulated [^{3}H]thymidine incorporation in MK cells. In one representative experiment MK cells treated with 20 ng/ml of epidermal growth factor incorporated 8146 ± 1322 counts/min (mean ± SD) of [^{3}H]thymidine compared with 836 ± 132 cpm in cells that did not receive epidermal growth factor.

Scatchard analysis of ^{125}I-EGF binding revealed the presence of high-affinity receptors in all five of the ovarian cancer cell lines (Fig. 3, *A* and *B*) and in the normal ovarian epithelial cells (Fig. 4, *A* and *B*). In the three normal epithelial cell cultures tested, linear Scatchard plots were obtained, which is indicative of a single class of high-affinity receptor (dissociation constant 0.61 to 1.42 nmol/L). Two of five ovarian cancer lines (OVCA 420 and DOV 13) appeared to have a single class of high-affinity epidermal growth factor receptor (dissociation constant 0.29 to 0.69 nmol/L), whereas the other three ovarian cancer cell lines appeared to have two classes of epidermal growth factor receptor of relatively lower and higher affinity (0.32 to 0.40 nmol/L; 1.11 to 1.39 nmol/L) (data not shown). The number of epidermal growth factor receptors per cell ranged from 1 to 1.4×10^5 in the normal ovarian cells and from 3.9×10^4 to 1×10^5 in the ovarian cancer cells (Table I). There was no relationship between epidermal growth factor receptor number or affinity and the effect of epidermal growth factor on [^{3}H]thymidine incorporation.

Comment

Epidermal growth factor is a potent mitogen for a wide range of normal cells in culture. In cells that are grown in culture medium that contains only small amounts of serum, epidermal growth factor usually acts to stimulate striking increases in deoxyribonucleic acid synthesis. In a prior study of human ovarian epithelial cells, Siemens et al.[8] showed that addition of epidermal growth factor to the culture media enhanced plating efficiency and growth. In the present study, we have demonstrated that normal human ovarian epithelial cells express high-affinity epidermal growth factor receptors and that epidermal growth factor consistently

Table I. Epidermal growth factor receptor number and affinity in normal ovarian epithelial cells and ovarian cancer cell lines

	*Effect of epidermal growth factor on [³H]thymidine incorporation**	*Epidermal growth factor dissociation constant (nmol/L)*	*Epidermal growth factor receptor sites (No./cell)*
Normal epithelium			
K.H.	++	1.42	1.4×10^5
C.L.	++	0.74	1.1×10^5
R.S.	−+	0.61	1.0×10^5
Cancer cell lines			
OVCA 420	+	0.23	3.9×10^4
OVCA 429	+	0.32/1.39	6.2×10^4
OVCA 432	−	0.31/1.23	5.1×10^4
OVCA 433	+	0.40/1.11	1.0×10^5
DOV 13	++	0.69	1.7×10^4

*−, No effect; ÷, 50% to 100% increase; ++, >200% increase.

acts to stimulate deoxyribonucleic acid synthesis in these cells. Although ovarian epithelial cells in vivo, for the most part, are quiescent cells that coat the ovarian surface, it is possible that epidermal growth factor may act to stimulate regrowth of epithelium after disruption of the ovarian surface at ovulation. Since it has been shown that epidermal growth factor is present in follicular fluid,[9] it is appealing to speculate that follicular cells secrete epidermal growth factor, which then acts in a paracrine fashion to stimulate growth of epithelium after rupture of the follicle.

Previously it has been shown that epidermal growth factor may act as a mitogen for various types of human cancer cells in culture.[10, 11] Among the five ovarian cancer cell lines that we studied, one increased proliferation markedly in response to epidermal growth factor (>twofold), three increased proliferation slightly (50% to 100%), and one was unaffected. In addition to our experiments that used immortalized cell lines, others have tested the effect of epidermal growth factor on growth of primary cultures of ovarian cancer cells. Among 16 gynecologic cancers studied by Singletary et al. (13 ovarian, 3 endometrial),[11] proliferation was increased by at least 100% in nine cases. This study, like ours, suggests that some, but not all, ovarian cancer cells are responsive to epidermal growth factor. Even in those cancer cells that respond to epidermal growth factor, however, the magnitude of the mitogenic response usually is less than that of normal cells.

Although several of the molecular events that occur following binding of epidermal growth factor to its receptor have been characterized, including tyrosine phosphorylation, intracellular alkalinization, calcium influx, and prostaglandin synthesis, the relationship between these events and deoxyribonucleic acid synthesis and cellular proliferation is not understood.[1, 2] It is clear, however, that the biologic effects of epidermal growth factor can be initiated only after binding of epidermal growth factor to its specific cell membrane receptor. It has been reported that a proportion of human breast,[4] ovarian,[12] and endometrial[13] cancers express little or no detectable epidermal growth factor receptor; therefore it has been proposed that the loss of responsiveness to epidermal growth factor of cancer cells in culture may be due to decreased receptor expression.

In normal ovarian epithelium, we found a single class of high-affinity binding sites for epidermal growth factor. The three normal ovarian epithelial cultures that were assessed had between 1 and 1.5×10^5 binding sites per cell. The ovarian cancer cell lines all had similar numbers of high-affinity receptors for epidermal growth factor as well. In three cancer cell lines linear Scatchard plots were obtained, indicating a single class of receptor, similar to the normal ovarian epithelial cells. In two cancer cell lines curvilinear Scatchard plots were obtained, consistent with two populations of receptors of different affinities. Although it has been postulated that the biologic effects of epidermal growth factor are mediated via the higher-affinity sites when two classes of receptor are present, this has not been proved and frequently cells that are responsive to epidermal growth factor have only a single class of receptor.[10, 14] Overall, the cancer cell lines had similar numbers of binding sites per cell as the normal ovarian epithelial cells. There was no relationship between epidermal growth factor receptor number or affinity and responsiveness to epidermal growth factor.

The results of this study are similar to those of others that have examined the relationship between epidermal growth factor receptor expression and response to epidermal growth factor in other types of cancers. For example, in a study in which four breast cancer cell lines were tested for mitogenic response to epidermal growth factor, only two were responsive despite the presence of epidermal growth factor receptors in all

cases.[10] In addition, Werner et al.[14] found variable responsiveness to epidermal growth factor in glioma cell lines. Proliferation of cell lines with 10^4 to 10^5 receptors per cell either increased or was unaffected by epidermal growth factor, whereas proliferation of cell lines with $>10^6$ receptors per cell was inhibited by epidermal growth factor. Similarly, epidermal growth factor inhibits proliferation of the A431 cell line, which markedly overexpresses the epidermal growth factor receptor.[15] None of the ovarian cancer cell lines that we have tested expressed such high levels of receptor nor was growth inhibition by epidermal growth factor observed.

The results of the present study indicate that the decreased mitogenic effect of epidermal growth factor in ovarian cancer cell lines relative to normal ovarian epithelium is not due to an absence of cell surface epidermal growth factor receptors. It is possible, however, that the attenuated response to epidermal growth factor could be due to alterations in the downstream signals involved in transmitting the mitogenic signal from the receptor to the nucleus. As these complex pathways are elucidated in the future, we will test their functional integrity in these ovarian cancer cell lines. Alternatively, it is possible that the ovarian cancer cell lines do not respond to exogenous epidermal growth factor because they already are maximally growth stimulated because of the production of other growth stimulatory factors or because of inactivation of tumor suppressor genes.

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Prenatal diagnosis of myotonic muscular dystrophy with linked deoxyribonucleic acid probes

Aubrey Milunsky, MB, BCh, DSc, DCH, James C. Skare, PhD, Jeffrey M. Milunsky, BA, Thomas A. Maher, BS, and Jean A. Amos, PhD

Boston, Massachusetts

Since the localization of the myotonic muscular dystrophy gene, closer deoxyribonucleic acid markers have been discovered. These now facilitate both presymptomatic and prenatal diagnosis of myotonic muscular dystrophy. We report our prenatal diagnosis experience with six cases in five families. Obstetricians are advised to inform their patients with a family history of myotonic muscular dystrophy of these testing opportunities. The fetus of the mother with myotonic muscular dystrophy who remains in utero until term is at considerable risk, as is the mother herself, of serious obstetric complications. (AM J OBSTET GYNECOL 1991;164:751-5.)

Key words: Myotonic muscular dystrophy, DNA, prenatal diagnosis

Myotonic muscular dystrophy is the most common form of muscular dystrophy in adults.[1] Women who are affected by this serious autosomal-dominant disorder may be free of symptoms or have minimal signs, yet they have a 50% risk of having a child with severe congenital myotonic muscular dystrophy. Indeed, although men and women with myotonic muscular dystrophy have the same 50% risk of transmitting it, the offspring of affected women are invariably more seriously affected.[1] Various other alternative mechanisms have been proposed, including mitochondrial inheritance, genetic imprinting, or a humoral factor.[1] Hence determination of a family history of myotonic muscular dystrophy will facilitate recognition of women at risk, albeit of those who are still free of symptoms. Since the localization of the myotonic muscular dystrophy gene to chromosome 19,[2] closer deoxyribonucleic acid (DNA) markers have been discovered.[3-7] These advances have facilitated the use of DNA probes for prenatal diagnosis of myotonic muscular dystrophy. DNA linkage studies in informative families provide results with high degrees (96% to 99%) of certainty, which are consistent with but not diagnostic of, myotonic muscular dystrophy. Thus far, only one team[8, 9] has reported on successful prenatal diagnosis of myotonic muscular dystrophy with these techniques. We report our experience with five families who sought prenatal studies through our DNA Diagnostic Laboratories, two of which were earlier noted by abstract.[10]

From the Center for Human Genetics and the Department of Pediatrics, Boston University School of Medicine.
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Reprint requests: Aubrey Milunsky, MD, Center for Human Genetics, Boston University School of Medicine, 80 East Concord St., Boston, MA 02118.
6/1/26389

Patients and methods

Families studied. Blood samples from key family members were sent to us by overnight express mail for DNA analysis from physicians elsewhere in the United States. When possible, family informativeness was determined before amniocentesis or chorionic villus sampling. Cultivation of amniotic fluid cells for about 14 days yielded sufficient DNA for molecular analysis. Chorionic villus sampling enabled direct DNA extraction and analysis with results within 10 days.

Pedigrees of the five families with myotonic muscular dystrophy that were studied are shown in Fig. 1; genotypes for each family member appear below each pedigree symbol. In accordance with Griggs et al.,[11] we confirmed that one or more individuals had been accurately characterized as having myotonic muscular dystrophy before linkage studies were undertaken.

Genetic analyses. Genomic DNA was prepared directly from peripheral lymphocytes and cultivated amniotic fluid cells by nuclear pellet isolation and lysis, protease digestion, phenol extraction, and ethanol precipitation.[12] Chorionic villi were lysed in a similar manner and processed as for blood. Restriction digestion and Southern blot analysis of restriction fragment length polymorphisms were performed with standard procedures with the probes shown in Table I.

Each available family member was studied with each of the probe/restriction combinations that are shown in Table I. Prenatal diagnoses were made with the closest restriction fragment length polymorphisms to the myotonic muscular dystrophy gene that was polymorphic in each family. The most likely order of the polymorphic loci that were used in these analyses and the myotonic muscular dystrophy locus is BCL3–ApoC2–CK MM–myotonic muscular dystrophy[5, 7]; BCL3 lies approximately 4 cM from the myotonic muscular dys-

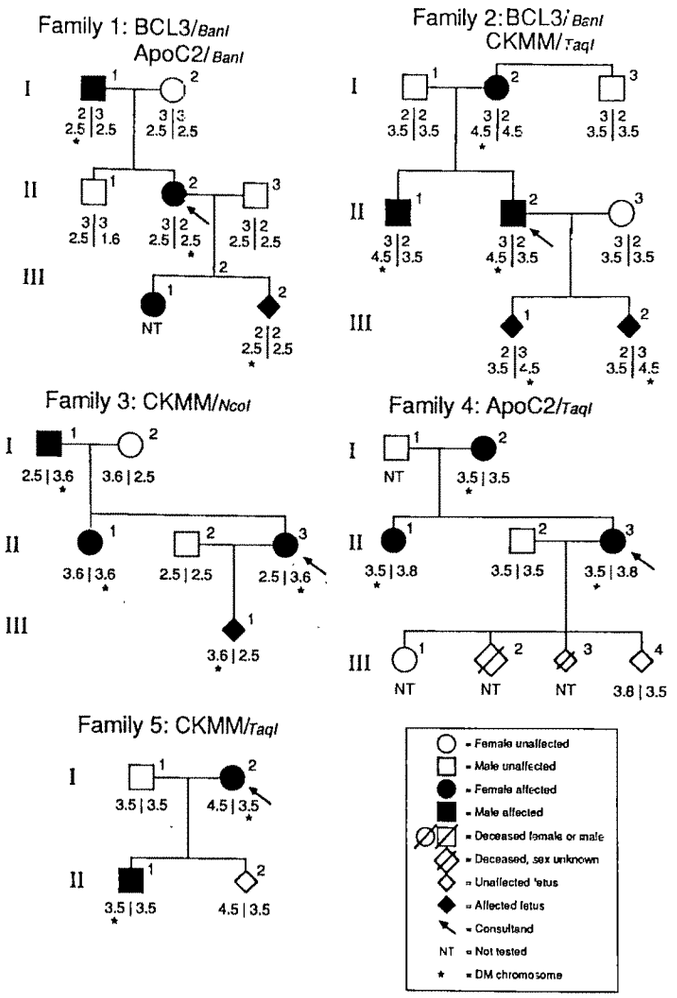


Fig. 1. Pedigrees and individual genotypes of five families who requested prenatal genetic studies.

trophy gene and ApoC2 and CK MM are both within 1 cM of the locus.

Plasmid DNA inserts were labeled by random primer synthesis with [^{32}P]αCTP, (3200 Ci/mmol) (Amersham) to a specific activity $>10^8$ cpm/μg.[13] Membranes were prehybridized, hybridized, washed, and exposed to x-ray film according to a method described elsewhere.[14]

Family 1. A mildly affected mother (II-2) with a previously affected child, aged 9 months (III-1), with joint contractures and developmental delay, elected to have prenatal diagnosis in her second pregnancy (Fig. 1). She declined our request for a blood sample from her first child. Earlier,[13] our DNA linkage data showed with a high degree of certainty that the proband (II-2) was affected, with the use of the Southern blot assay with BCL3/*Ban* I. With the same probe and enzyme, we then demonstrated that the fetus had inherited the maternal myotonic muscular dystrophy mutation with 96% certainty. In addition, nonpaternity for II-1 was discovered with ApoC2/*Ban* I.

The parents elected to continue this pregnancy, which was uneventful. Elective cesarean section was done because of a previous cesarean section. A daughter, who weighed 2980 gm, was delivered. Major respiratory difficulties were encountered immediately and Apgar scores were 5 at 1 minute and 6 at 5 minutes. Even at 30 minutes after birth, severe acidosis (pH 7.1) was present. Typical physical features of myotonic muscular dystrophy were noted and included a hanging open mouth, downturned palpebral fissures, a dull expression, absence of Moro, suck, and gag reflexes, extreme hypotonia with little limb movement, flexion of limb joints, bilateral talipes equinovarus, and ulnar deviation of the hands.

Recurrent episodes of apnea characterized the next 37 days in hospital, during which time mechanical ventilation was necessary. Complications included gastric hemorrhage, jaundice, and a patent ductus arteriosus, which required ligation. Gradual improvement occurred until 6 weeks after birth when the child was

discharged home with an apnea monitor. Prognosis for normal neurologic development was guarded.

Family 2. The consultand (II-2) and his wife requested prenatal diagnosis. His mother (I-2) had indisputable signs of myotonic muscular dystrophy, and he and his brother were considered to be affected in view of cataracts in both, which were diagnosed by examination with a slit lamp. Neither brother had experienced other symptoms or signs of myotonic muscular dystrophy. DNA analysis demonstrated that both sons (II-1 and II-2) had inherited the maternal myotonic muscular dystrophy mutation with 96% certainty, with the use of BCL3/*Ban* I. Prenatal diagnosis of an affected fetus was accomplished, after chorionic villus sampling for III-1, with the same probe and enzyme, as well as with CK MM/*Taq* I. The parents elected to terminate this pregnancy. No tissues were received for further study after termination. A second pregnancy was studied, and again a prenatal diagnosis of an affected fetus (III-2) was made. Once again, the parents elected to terminate this pregnancy. No tissue was received for further study.

Family 3. The consultand, who had symptoms (II-3), and her husband requested prenatal diagnosis. Earlier studies of her sister who had symptoms (II-1), her affected father (I-1), and the other family members showed that the consultand had inherited the same allele from her affected father. Prenatal diagnosis of myotonic muscular dystrophy for III-1 was made with CK MM/*Nco* I with 99% certainty. The parents elected to continue the pregnancy, and an affected child was born, as predicted.

After breech presentation, delivery by cesarean section because of intrauterine growth retardation and a known prenatal diagnosis of myotonic muscular dystrophy occurred without incident at 38 weeks' gestation. The female infant weighed 2200 gm and had Apgar scores of 5 at 1 minute and 7 at 5 minutes. Mild respiratory distress was detected immediately and successfully treated. She had myotonic facies, and very poor Moro, rooting, suck, and grasp reflexes. Marked hypotonia was evident. Diaphragmatic paralysis on the right side was demonstrated by chest x-ray films. Marked hyperbilirubinemia occurred but rapidly disappeared. Hospital discharge occurred at 1 week after birth. By 7 weeks of age, the child was doing well, had no real evidence of myotonia, was gaining weight, and had no pulmonary symptoms.

Family 4. The consultand (II-3) requested prenatal diagnosis for her fourth pregnancy. Her first pregnancy resulted in a girl with no congenital features of myotonic muscular dystrophy. Her second child died after aspiration of meconium 1 hour after birth. The third pregnancy ended in fetal death, possibly because of trisomy 13 syndrome. The consultand, her sister (II-1), and their mother (I-2) had clinical diagnoses of myotonic muscular dystrophy. Seven other siblings (not shown) had not been clinically evaluated and were not included in this analysis.

The informativeness study revealed that the myotonic muscular dystrophy allele in this family was transmitted with the maternal 3.5 kb ApoC2/*Taq* I allele. The consultand transmitted her paternal 3.8 kb allele to her fetus, which allowed us to predict that it would be unaffected with a certainty >98%. A healthy infant was delivered and DNA studies confirmed the prenatal diagnosis.

Family 5. The affected consultand (I-2) had previously had a son (II-1) with myotonic muscular dystrophy. In this, her second pregnancy she requested prenatal diagnosis at 22 weeks' gestation for late pregnancy management. The family study revealed that the myotonic muscular dystrophy allele was transmitted on the maternal chromosome 19 and bore the linked 3.5 kb CK MM/*Taq* I allele. The consultand transmitted her 4.5 kb allele to her fetus, which allowed a diagnosis of an unaffected child with >99% certainty. Pregnancy was uneventful, and a healthy daughter was delivered by cesarean section because of a previous cesarean section.

Comment

Myotonic muscular dystrophy was first detected prenatally, by means of genetic linkage with the secretor locus.[15, 16] This method was limited because of the few informative families, as well as because of the possibilities of missed diagnoses that were due to a recombination rate of approximately 10%. Assignment of the myotonic muscular dystrophy locus to chromosome 19 and the subsequent isolation of polymorphic, tightly linked DNA probes have now facilitated prenatal diagnosis with high degrees of accuracy. In this report we document the prenatal diagnosis of an affected fetus in four of six pregnancies at risk. Either chorionic villus sampling or amniocentesis provided adequate cellular samples. Regardless of tissue source, however, prenatal diagnostic studies should be attempted only after proper documentation of an affected family member has been established and formal genotypes of informative family members have been determined.

DNA studies provide a high degree of certainty that the myotonic muscular dystrophy gene has been inherited. This is especially important in a prospective mother and thereby alerts the obstetrician to probable serious maternal and perinatal complications (Table II).

All reviews of pregnancy with myotonic muscular dystrophy[1, 18-20] have pointed to an increased rate of complications. Many reports have involved single or few cases, and the real frequency of specific complications remains uncertain. However, a larger study by O'Brien and Harper[19] of 102 pregnancies among 35 affected women who had 83 infants has yielded a slightly better estimation of the rate of pregnancy com-

Table I. Probes used for studies of families with myotonic muscular dystrophy

Locus	*Probe*	*Enzyme*	*Allele length (kb)*	*Allele frequency*	*Recombination fraction*	*Reference no.*
BCL3	pα1.4p	*Ban* I	3.0	0.70	0.04	7
			2.2	0.30		
ApoC2	ApoC2	*Taq* I	3.8	0.55	0.01	16
			3.5	0.45		
CK MM	cCK MM3	*Taq* I	4.2	0.76	0.01	17
			4.3	0.24		
		Nco I	2.3 + 1.0	0.68		4
			3.3	0.32		

Table II. Myotonic muscular dystrophy: Pregnancy and neonatal complications[1, 16-20]*

1. Spontaneous abortion
2. Fetal death and stillbirth
3. Hydramnios
4. Premature labor
5. Prolonged labor
6. Fetal distress
7. Uterine atony
8. Postpartum hemorrhage
9. Retained placenta
10. Placenta previa
11. Elective cesarean section
12. Maternal cardiac arrhythmias
13. Increased maternal sensitivity to anesthetic and relaxant agents
14. Postoperative respiratory depression
15. Neonatal death
16. Arthrogryposis multiplex congenita

*Additional references are available from the authors on request.

plications when compared with normal pregnancies. Although an increased frequency of the complications that are listed in Table II may indeed occur in women with myotonic muscular dystrophy, thus far statistical significance can be claimed only for retained placenta, placenta previa, hydramnios, elected cesarean section, and neonatal death.[19] Hydramnios is thought to result from inadequate fetal swallowing in pregnancies in which the fetus has myotonic muscular dystrophy.

A prolonged first stage of labor, poor uterine contractions, and reduced voluntary muscle power in the second stage have all been cited.[1] Many authors have warned of major complications after general anesthesia or surgery, even when women with myotonic muscular dystrophy are mildly affected.[1, 19] Some authors have advised that general anesthesia be avoided when possible and have indicated that neostigmine, other cholinesterase inhibitors, and succinylcholine-like drugs, which may cause contractions, should not be used. Others have emphasized the intraoperative occurrence of cardiac arrhythmias during general anesthesia.[20] Use of thiopental may result in severe apnea in patients with myotonic muscular dystrophy.

Harper[1] has pointed to preeclampsia as the only complication for which frequency seems to be reduced in myotonic muscular dystrophy. This finding is probably due to the lowering of blood pressure in myotonic muscular dystrophy.

When parents are weighing the decision to have prenatal diagnostic studies performed or whether to abort an affected fetus, they will need to know about the increased frequency of mental retardation, progressive weakness and wasting, hypogonadism, and the increased mortality in young adulthood,[1, 19] particularly in the affected children of mothers with myotonic muscular dystrophy.

Obstetricians who have secured a prenatal diagnosis of myotonic muscular dystrophy in a continuing pregnancy are advised to have a neonatologist present for the delivery, given the known serious potential perinatal complications.

In families with a history of myotonic muscular dystrophy, presymptomatic testing, with molecular analysis as described, can now be offered. For those family members who are found to be affected, albeit without symptoms, prenatal diagnosis is now available with high degrees of certainty. Although elective abortion is one option, anticipation, prevention, or expeditious treatment will enable the physician to diminish the likelihood of maternal morbidity and neonatal morbidity and mortality if he or she has the results from presymptomatic, confirmatory, and prenatal diagnoses.

There are inherent limitations of DNA linkage analyses, and these are primarily (1) the possibility of recombination, (2) the inability to distinguish which chromosome of a pair contains the mutation, (3) only one affected family member, (4) deceased parents without a known diagnosis of myotonic muscular dystrophy, (5) unexpected discovery of nonpaternity, and (6) lack of family cooperation in providing medical data and/or blood samples. Notwithstanding such limitations, studies in our DNA diagnostic laboratories show that 17 of 19 (89.5%) families that were studied have been informative, and consultands have been able to obtain valuable information.

Development of even closer, flanking markers to the

myotonic muscular dystrophy locus will shortly further increase the accuracy of these studies. Application of the polymerase chain reaction technology will soon allow us to provide results within days of sample receipt.

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Addendum

We studied two additional families. In one, an affected fetus was detected, and pregnancy is continuing. In the other, the fetus was not affected by myotonic dystrophy but had hydrocephalus and other major anomalies at birth. Family 2 (Fig. 1) had a third pregnancy with twins, one being detected through analysis of chorionic villi as affected with >99% certainty. Selective termination was elected and pregnancy with the remaining fetus continues.

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Prevention of preterm birth in high-risk patients: The role of education and provider contact versus home uterine monitoring

Donald C. Dyson, MD,[a] Yvonne M. Crites, MD,[a] Deborah A. Ray, MD,[a] and Mary Anne Armstrong, MA[b]

Santa Clara and Oakland, California

A total of 394 patients were enrolled in a study to assess the effectiveness of an educational preterm delivery prevention program and to determine whether the addition of home uterine monitoring to the program improved results in patients at high risk of preterm labor. Both the educational program and home uterine monitoring were found to increase the percentage of women with preterm labor who sought care while still favorable for long-term suppression, resulting in a decreased incidence of preterm births and improved outcome when compared with similar high-risk patients who did not participate in these programs. In a randomized, prospective study, addition of home uterine monitoring to the educational program was found to significantly improve outcome in twin gestations but not in singleton gestations. However, the number of singleton pregnancies was too small to rule out possible benefit from home uterine monitoring in that group. (AM J OBSTET GYNECOL 1991;164:756-62.)

Key words: Premature labor, fetal monitoring, prenatal care, tocolysis

Preterm birth remains the main cause of perinatal mortality and morbidity.[1] Despite the increasing use of tocolytic agents, the incidence of preterm birth has remained unchanged.[2] The effect of tocolytic therapy has been severely limited because 70% to 80% of patients in whom preterm labor develops do not seek care until they have ruptured membranes or advanced cervical dilation, making long-term successful tocolysis unlikely.[3, 4] Recognition of the importance of early detection of preterm labor led to development of preterm delivery prevention programs that stressed identification of high-risk patients, patient education, self-palpation for uterine contractions, and frequent cervical examinations.[5] Although not uniformly successful,[6, 7] most studies have found this educational approach to be asssociated with a substantial increase in the percentage of patients with preterm labor who seek care while they are still favorable for suppression, with a resultant decrease in the incidence of preterm births.[5, 8-10]

From the Department of Obstetrics and Gynecology, Kaiser Permanente Medical Center, Santa Clara,[a] and the Division of Research, Kaiser Permanente Medical Care Program, Oakland.[b]
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Reprint requests: Donald C. Dyson, MD, Department of Obstetrics and Gynecology, Kaiser Permanente Medical Center, 900 Kiely Blvd., Santa Clara, CA 95051.
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Katz et al.[11] showed that overt clinical preterm labor is usually preceded by a period of increased uterine activity that is readily detected by tocodynamometry. Subsequent studies showed that daily home monitoring of uterine activity in high-risk patients also appears effective in increasing the proportion of patients with preterm labor who seek care while they are favorable for suppression, with a resultant decrease in the incidence of preterm births.[4, 12] However, other studies[13, 14] questioned whether it was the monitoring, data from the monitoring, or the patient education and nursing contact that was provided with the monitoring service that was responsible for the improved outcome. The purpose of the present investigation is twofold: (1) to document whether implementation of an organized preterm delivery prevention program results in an improved outcome in patients at high risk of preterm labor, and (2) with the use of a randomized, prospective design, to assess whether the addition of home uterine monitoring to the preterm delivery prevention program would further improve outcome.

Material and methods

Between Jan. 1, 1986, and Jan. 1, 1989, patients who received prenatal care at the Kaiser Permanente Medical Center, Santa Clara, Calif., were prospectively assessed for the following risk factors: twin gestation, preterm delivery at <34 weeks' gestation after premature labor or premature rupture of membranes in the preceding pregnancy, premature labor requiring parenteral tocolysis but with term delivery in the preceding pregnancy, incompetent cervix with cerclage in place, and nullipara with bicornuate, subseptate, or di-

delphic uterus. A total of 253 patients with these risk factors identified before 28 weeks' gestation and without evidence of preterm labor in the current pregnancy, were asked to participate in this study. Two patients refused and 251 patients (99%) gave informed consent and were enrolled. At 24 weeks' gestation, all patients received an initial educational session concerning the signs and symptoms of preterm labor, were taught the technique of self-palpation for uterine activity, and were followed up with weekly cervical examinations. All patients were provided monitors (DT-100, Advanced Medical Systems, Hamden, Conn.) for home uterine monitoring. According to the protocol approved by the Institutional Review Board of the Kaiser Foundation Research Institute, patients were randomized into two groups: the home uterine monitoring group, in which the home uterine monitoring tracings were analyzed and used in patient management, and education-palpation group, in which home uterine monitoring tracings were not analyzed or used in patient management. Patients in the education-palpation group routinely transmitted to a monitor in such a way that the nurse could tell which patients had transmitted but could not analyze uterine activity data. On rare occasions, patients in the education-palpation group erroneously transmitted to a different monitor. When this occurred, the protocol was for the nurse to not analyze or respond to the tracing. The charts of all patients in the education-palpation group who experienced preterm labor were reviewed, and in no case did it appear that a patient in the education-palpation group was referred by a nurse for increased uterine activity detected by one of these accidentally unblinded tracings. Patients were not aware of their group assignments. All patients were initially asked to monitor themselves 1 hour every day and transmit daily. On Jan. 1, 1988, the protocol was changed to twice-daily monitoring and transmission, and patients in the home uterine monitoring group were given the capability of transmitting an emergent tracing at any time. All patients were asked to record the presence or absence of signs and symptoms of preterm labor and the number of contractions by palpation.

All patients were contacted at least 5 days per week by a study nurse to elicit signs or symptoms of preterm labor, record the number of contractions by palpation, and, in the home uterine monitoring group, to review the monitoring information. All patients were instructed to report to the hospital for evaluation if they had more than five contractions per hour that persisted for more than 1 hour despite lateral bed rest and oral hydration. Asymptomatic patients in the home uterine monitoring group who had more than five contractions per hour on routine monitoring were instructed to lie down, drink fluids, and remonitor for 1 hour. If they persisted with more than five contractions per hour they were referred for evaluation.

All patients in this study were evaluated and treated at Kaiser Foundation Hospital, Santa Clara, Calif., with clinical protocols that recommended tocolysis for persistent uterine contractions if the cervix was already dilated to 2 cm but otherwise required documented cervical change before tocolysis. Prophylactic tocolytic agents were not administered to any patients in this study. Choice of tocolytic agents used was determined by clinical protocol and physician preference and was neither affected by nor did it differ by study groups. If tocolysis was successful, patients were discharged home to bed rest taking oral tocolytic agents and maintaining their pretreatment group protocols. In the home uterine monitoring and education-palpation groups, the dosage of oral tocolytic agent was adjusted to maintain fewer than six contractions per hour on monitoring and on palpation, respectively. Oral tocolysis and monitoring were discontinued at 36 weeks' gestation. Patients who were delivered of infants at <36 weeks' gestation were classified as either a result of failed tocolytic therapy or as indicated preterm births. All gestational ages were confirmed by first- or second-trimester ultrasonography.

A group of 143 patients with the same risk factors for preterm labor who received their prenatal care and were delivered of infants at the same hospital in the 30 months immediately preceding the start of the prospective study was identified as the standard care group. To avoid adverse selection, particular care was taken to select patients by the presence of the risk factor and not the outcome. The same clinical protocols for assessment and treatment of preterm labor were in effect during both the control and study time periods. In this study, preterm labor was diagnosed only if there were persistent uterine contractions (>5 hours) associated with either 2 cm of cervical dilation or documented cervical changes. Favorable for suppression was defined as intact membranes and cervix dilated ≤2 cm. Respiratory distress syndrome was diagnosed if there was a neonatal oxygen requirement that lasted >24 hours and a compatible x-ray film of the chest. Statistical analysis was performed with the χ^2 test, the Fisher exact test, or the Student t test, as appropriate. Results are expressed as mean ± SEM. All p values of ≤0.10 were reported in the tables.

Results

A total of 394 patients were enrolled in the study. Four patients (1.6%), two patients each from the home uterine monitoring and education-palpation groups, subsequently elected to drop out of the study, resulting in a total of 247 patients in the randomized study and 143 in the control group. Of these, 189 were twin ges-

Table I. Pregnancy outcome: singleton gestations

Outcome	*SC* (%)*	*EP† (%)*	*HUM‡ (%)*	*SC vs EP (p value)*	*SC vs HUM (p value)*	*HUM vs EP (p value)*
PTL <36 wk	28.6	34.3	33.8	NS	NS	NS
PTL <34 wk	20.6	27.1	27.9	NS	NS	NS
PTB <36 wk	31.8	20.0	23.5	0.09	NS	NS
PTB <36 wk, FT	25.4	5.7	13.2	<0.01	0.06	NS
PTB <34 wk	23.8	11.4	11.8	0.05	0.06	NS
PTB <34 wk, FT	20.6	5.7	7.4	0.01	0.03	NS

EP, Education-palpation group; *FT,* failed tocolysis; *HUM,* home uterine monitoring group; *NS,* not significant; *PTB,* preterm birth; *PTL,* preterm labor; *SC,* standard care group.

*$n = 63$.

†$n = 70$.

‡$n = 68$.

Table II. Neonatal outcome: singleton gestations

Outcome	*SC (n = 63)*	*EP (n = 70)*	*HUM (n = 68)*	*SC vs EP (p value)*	*SC vs HUM (p value)*	*HUM vs EP (p value)*
Birth weight (gm)*	2767 ± 109	2882 ± 94	2938 ± 93	NS	NS	NS
<2500	31.8%	18.6%	20.6%	0.06	0.10	NS
<1500	7.9%	8.6%	5.9%	NS	NS	NS
RDS	14.5%	6.0%	8.8%	0.09	NS	NS
ICN admission	29.0%	16.4%	22.1%	0.07	NS	NS
IHS (days)*	10.9 ± 2.3	5.2 ± 1.4	8.8 ± 2.5	0.04	NS	NS

EP, Education-palpation group; *HUM,* home uterine monitoring group; *ICN,* intensive care nursery; *IHS,* infant hospital stay; *RDS,* respiratory distress syndrome; *SC,* standard care group.

*Mean ± SEM.

tations and 201 were singleton gestations. Analysis by risk factor in patients with singleton gestations revealed no statistically significant differences in outcome; however, significant differences were found between patients with singleton gestations and those with twin gestations. Results are therefore presented separately for singleton and twin gestations.

Singleton gestations. The distribution of risk factors for preterm labor was similar among all three groups of patients. One previous preterm birth was the indication in 63% of the standard care group, 65% of the education-palpation group, and 56% of the home uterine monitoring group; two or more previous preterm births was the indication in 17%, 16%, and 23% in the standard care, education-palpation, and home uterine monitoring groups, respectively. The mean number of unscheduled visits to rule out preterm labor was greater in both the education-palpation (1.4) versus the standard care group (0.2, $p = 0.01$) and in the home uterine monitoring (1.8) versus the standard care group (0.2, $p = 0.01$). The difference between the education-palpation and home uterine monitoring groups was not statistically significant. As shown in Table I, the difference in incidence of preterm labor was not statistically significant when comparing the three groups in pairs; however, the incidence of preterm birth was markedly less in the education-palpation group than in the standard care group. The incidence of preterm birth also appears to be less in the home uterine monitoring group than in the standard care group, but the difference reached statistical significance only for those delivered of infants at <34 weeks' gestation, specifically because of failed tocolysis. There was no apparent difference between the education-palpation and home uterine monitoring groups. Table II shows that although there were definite trends toward higher birth weights, shorter infant hospital stays, fewer infants admitted to the intensive care nursery, and fewer infants who developed respiratory distress syndrome in both the education-palpation and home uterine monitoring groups when compared with the standard care group, only the shorter infant stay in the standard care versus education-palpation comparison reached statistical significance.

In all three groups, patients with preterm labor at <34 weeks' gestation had aggressive tocolytic therapy unless there were contraindications. Management of patients with onset of preterm labor between 34 and 36 weeks' gestation was less uniform and was characterized by less aggressive use of tocolytic agents and occasionally nontreatment, especially after 35 weeks' gestation. Outcome data are thus reported for both 34 and 36 weeks' gestation. Table III shows indicators of pregnancy outcome in only those patients with onset of pre-

Table III. Patients with singleton gestations with preterm labor <34 weeks' gestation

Outcome	*SC (n = 13)*	*EP (n = 19)*	*HUM (n = 19)*	*SC vs EP (p value)*	*SC vs HUM (p value)*	*HUM vs EP (p value)*
GA at PTL (wk)*	29.7 ± 0.9	29.1 ± 0.6	29.1 ± 0.7	NS	NS	NS
GA at delivery (wk)*	30.8 ± 0.6	35.5 ± 0.9	34.7 ± 0.9	<0.01	<0.01	NS
Days gained*	7.3 ± 3.6	44.7 ± 6.9	39.2 ± 5.6	<0.01	<0.01	NS
Favorable for suppression	30.8%	84.2%	68.4%	<0.01	0.04	NS
Delay >48 hr	46.2%	89.5%	89.5%	0.01	0.01	NS
Delivered >34 wk	0	73.7%	68.4%	<0.01	<0.01	NS
ICN admission	92.3%	36.8%	47.4%	<0.01	0.01	NS
RDS	69.2%	21.1%	26.3%	0.01	0.02	NS
BWT <2500 gm	100%	36.8%	47.4%	<0.01	<0.01	NS
BWT (gm)*	1566 ± 110	2624 ± 211	2299 ± 174	<0.01	<0.01	NS
IHS (days)*	36.4 ± 7.5	10.5 ± 5.0	16.9 ± 6.0	0.01	0.05	NS

BWT, Birth weight; *EP,* education-palpation group; *GA,* gestational age; *HUM,* home uterine monitoring group; *IHS,* infant hospital stay; *PTL,* preterm labor; *RDS,* respiratory distress syndrome; *SC,* standard care group.

*Mean ± SEM.

Table IV. Pregnancy outcome: twin gestations

Outcome	*SC* (%)*	*EP† (%)*	*HUM‡ (%)*	*SC vs EP (p value)*	*SC vs HUM (p value)*	*HUM vs EP (p value)*
PTL <36 wk	45.0	42.1	48.1	NS	NS	NS
PTL <34 wk	32.5	36.8	42.3	NS	NS	NS
PTB <36 wk	46.3	29.8	23.1	0.05	0.01	NS
PTB <36 wk, FT	26.3	14.0	7.7	0.06	0.01	NS
PTB <34 wk	28.8	17.5	9.6	NS	0.01	NS
PTB <34 wk, FT	22.5	10.5	5.8	0.05	0.01	NS

EP, Education-palpation group; *FT,* failed tocolysis; *HUM,* home uterine monitoring; *NS,* not significant; *PTB,* preterm birth; *PTL,* preterm labor; *SC,* standard care group.

*$n = 80$.

†$n = 57$.

‡$n = 52$.

term labor at <34 weeks' gestation. A much higher proportion of patients in the education-palpation and home uterine monitoring groups were seen while still favorable for suppression, with a resultant improvement in success rate of both short-term (>48 hours) and long-term (delivery after 34 weeks' gestation) suppression. Correspondingly, neonatal outcome as measured by birth weight, percent with intensive care nursery admission or respiratory distress syndrome, and duration of infant hospital stay all were significantly improved in both the home uterine monitoring and education-palpation groups as compared with the standard care group. There was no apparent difference between home uterine monitoring and education-palpation groups in any of the factors in Table III.

Twin gestations. There were no statistically significant differences between groups in the patient demographics including maternal age, gravidity, parity, percent nulliparous, and percent with previous preterm delivery. Table IV shows that whereas the incidence of preterm labor was similar in all three groups, the incidence of preterm birth was decreased in the education-palpation group and markedly decreased in the home uterine monitoring group compared with the standard care group. The incidence of patients delivered of premature infants as a result of failed tocolytic therapy at <34 weeks' gestation was decreased by 53% in the education-palpation group and by 74% in the home uterine monitoring group as compared with the standard care group ($p = 0.05$ and 0.01, respectively). Table V shows that infants in the education-palpation group tended to have a better outcome than those in the standard care group and were significantly less likely to be admitted to the intensive care nursery, develop respiratory distress syndrome, and experience neonatal death than were the infants in the standard care group. Infants in the home uterine monitoring group clearly had the best neonatal outcome, having shown highly significant improvements in all indicators of neonatal outcome as compared with the infants in the standard care group and were significantly less likely to have weighed <1500 gm, to have been admitted to the intensive care nursery, and to have had a shorter hospital stay as compared with the infants in the education-palpation group.

Table VI shows that patients with twin gestations in whom the onset of preterm labor developed before 34 weeks' gestation had similar gestational ages when that

Table V. Neonatal outcome: twin gestations

Outcome	*SC (n = 160)*	*EP (n = 114)*	*HUM (n = 104)*	*SC vs EP (p value)*	*SC vs HUM (p value)*	*HUM vs EP (p value)*
BWT (gm)*	2333 ± 83	2535 ± 87	2687 ± 71	0.09	0.00	NS
BWT <2500 gm	52.2%	46.5%	36.5%	NS	0.01	0.09
BWT <1500 gm	14.5%	8.8%	1.9%	NS	0.00	0.03
RDS	21.9%	8.8%	3.9%	0.00	0.00	0.10
ICN admission	57.5%	43.9%	27.9%	0.02	0.00	0.01
Neonatal death	7.5%	0.9%	0	0.01	0.00	NS
IHS (days)*	11.7 ± 1.4	10.2 ± 1.7	5.6 ± 0.4	NS	0.00	0.01

BWT, Birth weight; *EP,* education-palpation group; *HUM,* home uterine monitoring group; *ICN,* intensive care nursery; *IHS,* infant hospital stay; *RDS,* respiratory distress syndrome; *SC,* standard care group.

*Mean ± SEM.

Table VI. Patients with twin gestations with preterm labor <34 weeks' gestation

Outcome	*SC*	*EP*	*HUM*	*SC vs EP (p value)*	*SC vs HUM (p value)*	*HUM vs EP (p value)*	*SC vs HUM + EP (p value)*
Maternal	(n = 26)	(n = 21)	(n = 22)				
GA at PTL (wk)*	30.2 ± 0.7	30.0 ± 0.6	31.1 ± 0.5	NS	NS	NS	NS
GA at delivery (wk)*	31.1 ± 0.9	33.9 ± 0.8	36.1 ± 0.5	0.02	0.00	0.03	0.00
Days gained*	6.0 ± 2.2	27.4 ± 4.9	34.6 ± 4.3	0.00	0.00	NS	0.00
Favorable for suppression	26.9%	66.7%	95.5%	0.01	0.00	0.02	0.00
Delay >48 hr	34.6%	76.2%	100.0%	0.01	0.00	0.02	0.00
Delivered >34 wk	19.2%	52.4%	81.8%	0.02	0.00	0.04	0.00
Neonatal	(n = 52)	(n = 42)	(n = 44)				
ICN admission	88.2%	70.7%	41.9%	0.03	0.00	0.01	0.00
RDS	50.9%	24.4%	9.3%	0.01	0.00	0.06	0.00
BWT <1500 gm	40.4%	23.8%	4.6%	0.07	0.00	0.01	0.00
BWT (gm)*	1700 ± 106	2098 ± 112	2476 ± 83	0.01	0.00	0.01	0.00
IHS (days)*	22.6 ± 3.7	19.6 ± 4.4	6.8 ± 0.9	NS	0.00	0.00	0.03

EP, Education-palpation group; *GA,* gestational age; *HUM,* home uterine monitoring group; *ICN,* intensive care nursery; *IHS,* infant hospital stay; *RDS,* respiratory distress syndrome; *SC,* standard care group.

*Mean ± SEM.

occurred. However, gestational age at delivery was significantly improved in both the education-palpation and home uterine monitoring groups as compared with the standard care group. Once again, this appeared to be a result of a higher percentage of patients who were seen while still favorable for suppression, resulting in improved short-term and long-term tocolysis and improved neonatal outcome in both the education-palpation and home uterine monitoring groups. Table VI also shows that whereas both the education-palpation and home uterine monitoring groups showed an improved outcome compared with the standard care group, the home uterine monitoring group had the best outcome; the home uterine monitoring group had a statistically significant improvement in the percentage of patients seeking care while still favorable for suppression, in effectiveness of short-term and long-term tocolysis, and in all indicators of neonatal outcome as compared with the education-palpation group.

Comment

The success of tocolytic therapy is highly dependent on the status of the cervix and membranes at the initiation of tocolysis.[15] Early detection and treatment of preterm labor has been advocated as the key factor in the prevention of preterm births.[5] Unfortunately, studies have suggested that 70% to 80% of women in whom preterm labor develops are seen only after they have ruptured membranes or have advanced cervical dilation, making successful longer-term tocolysis unlikely.[3, 4] Our study confirms these findings, with 69% of women with singleton pregnancies and 73% of those with twin gestations in our standard care group who experienced preterm labor seeking care when they were no longer favorable for long-term tocolytic therapy.

Our study agrees with previous reports[5, 8-10] that suggest that participation in an organized preterm delivery prevention program that emphasizes patient education and frequent provider contact can significantly increase the percentage of high-risk patients in whom preterm labor develops who seek care while still favorable for suppression, thus increasing the success of tocolysis and decreasing the incidence of preterm birth. In our study, the percentage of women with preterm labor who sought care while still favorable for suppression increased from 31% to 84% in singleton and from 27%

to 68% in multiple gestations after initiation of such a preterm delivery prevention program (education-palpation group), and the incidence of preterm birth at <34 weeks' gestation subsequently decreased by 54% in singleton gestations and by 41% in twins.

Our study also agrees with previous reports[4, 12] that suggest that daily home monitoring of uterine activity in high-risk patients can also increase the early detection of preterm labor, allowing more successful treatment and improved outcome. In our study, the percentage of patients with preterm labor who sought care while still favorable for suppression increased from 31% to 68% in singleton and from 27% to 96% in multiple gestations in the home uterine monitoring group, and the incidence of preterm birth at <34 weeks' gestation decreased by 50% in singleton and by 66% in multiple gestations, resulting in a highly significant improvement in all indicators of neonatal outcome in the home uterine monitoring group compared with the standard care group.

Our study strongly suggests that both the educational approach and home uterine monitoring are effective in the improvement of outcome in patients at high risk of preterm labor. However, in the home uterine monitoring group, the question[14] as to whether it is the monitoring information or the frequent nursing contact that occurs in conjunction with home uterine monitoring that results in improved outcome is more difficult to answer. In singleton pregnancies, within the limits of our study design, there was no apparent difference in outcome between the education-palpation and home uterine monitoring groups. However, in patients with twin gestations, use of home uterine monitoring information resulted in an increase in those with preterm labor favorable for suppression from 68% to 96% and an increase in the success of both short-term (76% to 100%) and long-term (52% to 82%) tocolysis compared with the education-palpation group. Although the 45% reduction in preterm births before 34 weeks' gestation did not reach statistical significance, there was a significant reduction in both births of <1500 gm and admissions to the intensive care nursery as well as shorter infant hospital stays in the home uterine monitoring group compared with the education-palpation group. Thus our study suggests that for patients with a twin gestation, the addition of home uterine monitoring information will improve outcome even if they are already participating in an intense preterm delivery prevention program with frequent provider telephone contact.

There are several possible explanations as to why home uterine monitoring was found to be more beneficial in twin gestations than in singleton gestations in this study. It has been reported that patients with multiple gestations perceive contractions less accurately than do patients with singleton gestations.[16] In multiple gestations, this decreased perception may limit the effectiveness of programs that depend on the mother's ability to perceive or detect contractions. The possible bias of nurses who have occasional accidental knowledge of home uterine monitoring data in education-palpation patients is an unlikely explanation of the failure to find a difference in singleton gestation because the same nursing protocols were used with twin gestations in which significant improvement was found with home uterine monitoring. The use of six contractions per hour as the threshold, instead of the more commonly used four contractions per hour, is also an unlikely explanation of the failure to find efficacy in home uterine monitoring in singletons because only one patient in the singleton home uterine monitoring group was found to have had between four and six contractions per hour on the last tracing before diagnosis of preterm labor. However, the design of our study may have acted to minimize the differences in outcome between the home uterine monitoring and education-palpation groups. Patients in the education-palpation group were contacted at least 5 days per week by telephone. This is a much higher standard of care than that found in most preterm delivery prevention programs. Patients in the education-palpation group also received a home monitoring device. Although the monitoring information was not used, presence of a monitor may well have decreased the dropout rate and increased the compliance in the education-palpation group. In addition, the lower rate of preterm labor and the relatively small number of patients in our singleton groups would make it more difficult to detect a true difference. With the size of our singleton groups, to have a power of 80% would require a 75% or larger reduction in preterm births in the home uterine monitoring group (20% to 5%), with a two-tailed hypothesis test and a significance level of 0.05. Our numbers are thus too small to reasonably expect to detect a reduction in preterm labor as a result of uterine monitoring of <75% in our singleton groups.

In summary, our patient population consists predominantly of compliant patients from the middle socioeconomic class with a relatively low incidence of drug abuse and infection complications, so that these interventions may not be as successful in other dissimilar patient populations. In our study, both an organized preterm delivery prevention program with the use of frequent telephone contact with nurses and a home uterine monitoring program were found to significantly improve outcome in patients at high risk of preterm labor compared with our previous standard of care. In our study, the addition of home uterine monitoring in patients with twin gestations resulted in improved outcome as compared with those patients man-

aged with frequent telphone contact with nurses only. The issue of efficacy of home uterine monitoring in singleton pregnancies at high risk of preterm labor remains unresolved and awaits further clinical trials. However, our own internal cost analysis suggests that providing the same intense preterm delivery prevention program with frequent nursing contact but deleting the home uterine monitor would result in only a 17% cost saving. Thus on the basis of our study and others,[5, 8-10] it appears that patients with singleton gestations who have major risk factors of preterm labor benefit greatly from participating in a preterm delivery prevention program. If the components of a preterm delivery prevention program (patient education and frequent provider contact) cannot otherwise be made available to these high-risk patients, which is often the case in clinical practice, then home uterine monitoring may be a reasonable alternative. From our study, it also appears that home monitoring of uterine activity is the most effective method to decrease the preterm birth rate in twin gestations. Whereas the increased use of these intense intervention programs should substantially decrease the preterm birth rate in high-risk patients, our ability to minimize the overall preterm birth rate will continue to be limited as long as 40% to 60% of preterm births continue to occur in patients with no identifiable risk factors for preterm labor.[5, 6]

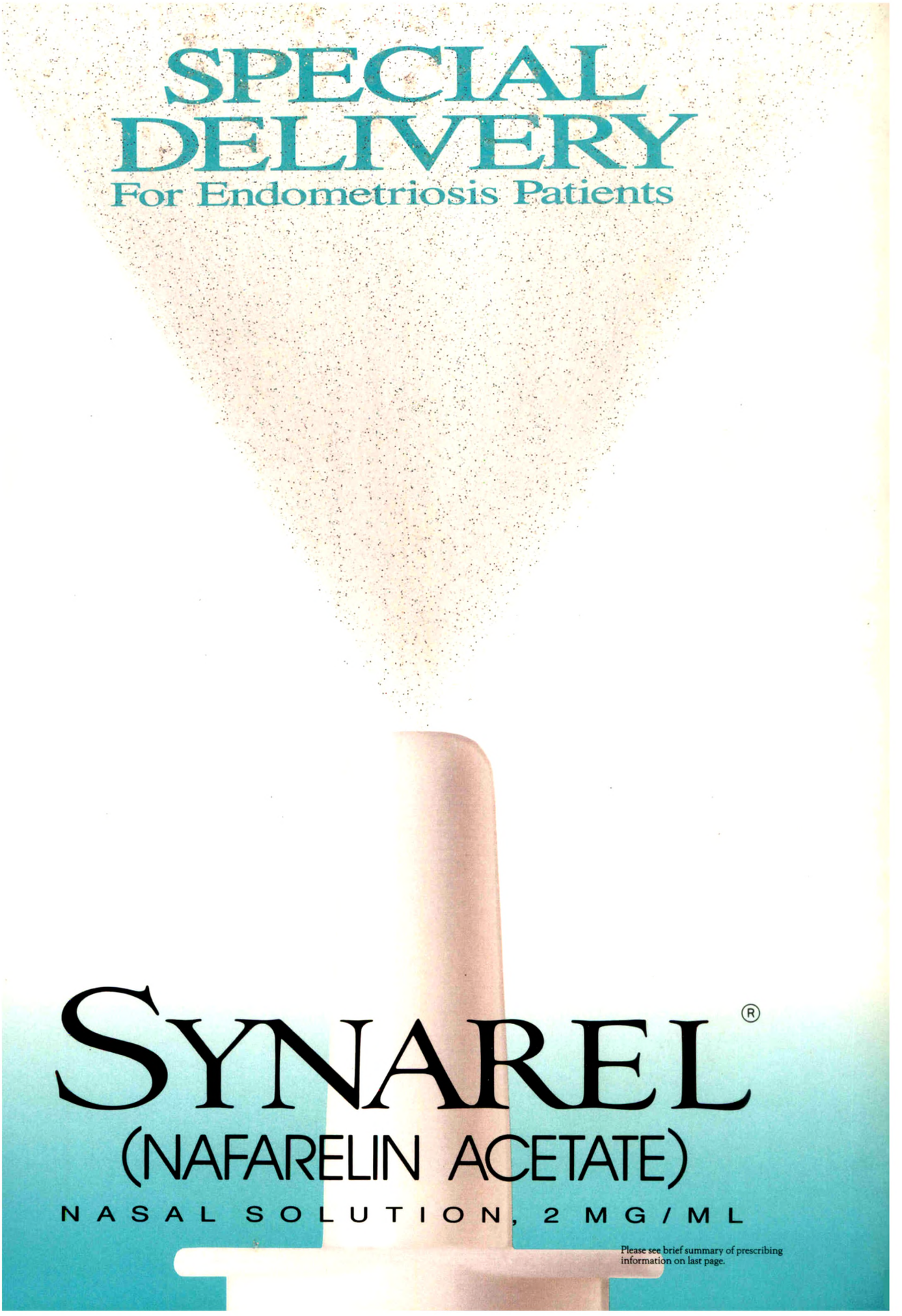
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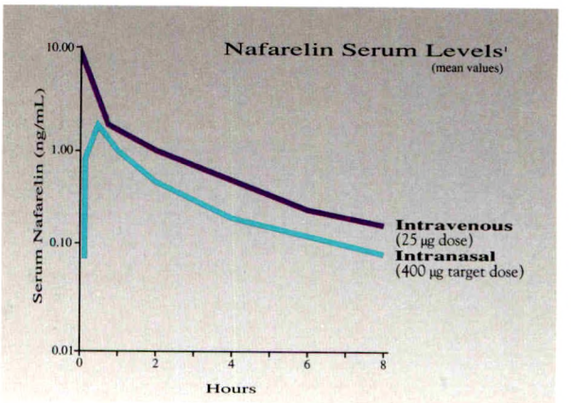
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BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloride, glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.

INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.

CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.

WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.

When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.

PRECAUTIONS Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. Patients missing successive doses may experience breakthrough bleeding.

2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients.

3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician.

4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients.

5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content.

6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption.

7. Retreatment cannot be recommended since safety data beyond 6 months are not available.

Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.

Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.

Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.

Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (±SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

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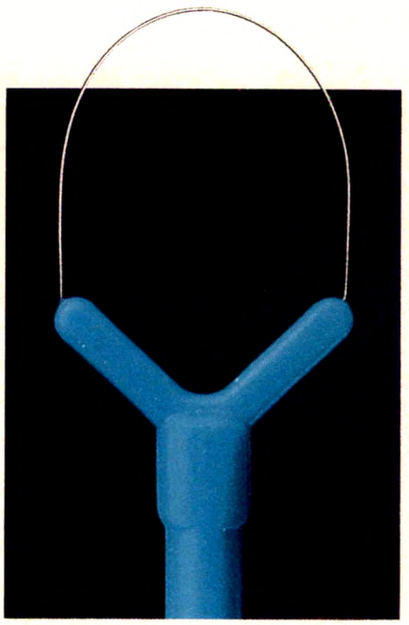
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Impact of tubal sterilization and vasectomy on female marital sexuality: Results of a controlled longitudinal study

Rochelle N. Shain, PhD,[a] Warren B. Miller, MD[b] Alan E.C. Holden, MA,[a] and Marc Rosenthal, MD, PhD[a]

San Antonio, Texas, and Palo Alto, California

To determine if female or male sterilization affects long-term female marital sexuality, we prospectively compared baseline data and 5 consecutive years of follow-up data from 152 tubal sterilization women, 106 vasectomy wives, and 83 women not planning sterilization. By follow-up year 5, no group of women expressed any change in satisfaction with their own sexual response; however, all groups showed a significant decrease across time in satisfaction with their sexual relationship, in coital desire, and in coital frequency. There were no group differences in overall net changes or in rates of change over the 5-year period. However, two short-term group differences were noted: a decrease in coital desire among women not planning sterilization between baseline and follow-up year 4, compared with increases for both sterilization groups, and an increase in coital frequency at the first follow-up year only in the tubal sterilization group. These data indicate that there are no detrimental effects and some short-term benefits of both sterilization procedures on female marital sexuality. (AM J OBSTET GYNECOL 1991;164:763-71.)

Key words: Female marital sexuality, change in sexuality, tubal sterilization, vasectomy

Surgical sterilization has become the most common contraceptive method for currently or previously married women in the United States.[1] As of 1984 approximately 17,700,000 (8,050,000 male and 9,650,000 female) sterilizations had been performed in the United States.[2,3] Estimates are not available for 1985 or 1986. During 1987, however, the Association for Voluntary Surgical Contraception[4] estimates that an additional 640,000 female and 336,000 male procedures were performed; moreover, other sources[5] indicate that in 1987, 51% of married women who were neither pregnant, trying to conceive, nor infertile, and who were using contraception, were protected by male or female sterilization. The popularity of these procedures is not confined to the United States. International estimates made in 1982 and 1984 indicate that approximately 33 million couples are protected by vasectomy[6] and 95 million by tubal sterilization.[7]

Despite the worldwide popularity of female and male sterilization, there are large gaps in our knowledge regarding long-term psychosocial and physical sequelae.[8-14] Of particular interest is long-term change in female sexuality, primarily coital frequency, sexual desire, and sexual satisfaction. Deterioration in some aspect of sexuality after female sterilization is reported by 2% to 28% of women, whereas improvements range between 0% and 55%.[10,12] In most studies the majority of respondents report no change or improvements.[10,12] Both positive and negative changes have been reported within the same study, as might be expected, because sterilization can have different psychological effects on different people. For example, positive effects may result from liberation from fears of pregnancy and negative effects from associating desirability with reproductive integrity.

Several studies compared sterilized women with users of nonpermanent contraceptive methods or with women married to vasectomized men. In a prospective British study, women who had undergone tubal sterilization showed greater improvement in overall sexual relationships than did users of nonpermanent methods at 1 year after the procedure. The latter, in fact, reported less satisfaction than they reported in baseline interviews.[15] Two retrospective investigations at 6 months'[16] and 2 years'[17] follow-up suggested that wives of vasectomized men showed greater increases in sexual desire[16] and had higher rates of coital frequency[17] and fewer sexual problems[17] than did sterilized women.

A few studies considered sexual change at more than one time point after sterilization. In a prospective, but uncontrolled British investigation,[18] women who had tubal sterilization experienced an increase in coital frequency at 6 months after the procedure that disappeared by 18 months. Married women included in both

From the Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio,[a] and Transnational Family Research Institute, Palo Alto.[b]
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Reprint requests: Rochelle N. Shain, PhD, Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284.
6/1/26283

the 1970 and 1975 National Fertility Studies, who were initially sterilized or who became sterilized during the interval, experienced smaller (statistical significance is not addressed) declines in coital frequency than did users of other methods, including vasectomy.[19]

Whereas prior studies have made valuable contributions, results are inconsistent and do not allow rigorous assessment of sterilization's impact on sexuality, especially as it changes over time. Limitations include retrospective design, subjective assessment of change, short follow-up periods, very small sample size, examination of limited aspects of sexual behavior, lack of independent controls, and/or failure to control for factors that may independently affect sexuality.

This article compares changes in coital frequency, coital desire, and satisfaction with sexual relationship and personal sexual response across 5 years of follow-up among sterilized women, women married to vasectomized men, and women who had completed childbearing but did not plan sterilization. These groups are subsequently referred to as tubal sterilization women, vasectomy wives, and women not planning sterilization. On the basis of the literature, we hypothesized that female sterilization and vasectomy would have no effect, or perhaps a small positive effect, on sexuality.

Material and methods

Sample selection. Subjects are non-Hispanic, white women between 19 and 49 years of age, chosen from patients in approximately 50 obstetrics and gynecology and urology offices and clinics in San Antonio, serving lower-, middle-, and upper-class individuals. All protocols received institutional review board approval. Women in the tubal sterilization group were recruited through a letter given to them in their physicians' offices. Space was provided for names and telephone numbers of patients who agreed to be contacted. Women who did not permit further contact were requested to provide (anonymously) basic sociodemographic information. Women accompanying their husbands to urology offices for preoperative vasectomy counseling or inquiries were also given recruitment letters. Men who were not accompanied by spouses were asked to release their wives' names and phone numbers. Trained interviewers followed up with telephone contact, at which time additional information about the study was provided and an interview was scheduled. Women who reported that tubal sterilization or vasectomy was being performed solely because of the wife's medical status were excluded.

Women not planning sterilization were recruited in various ways, primarily through distribution of recruitment letters, recruitment in physicians' offices by study personnel, and physician-staff searches of patient files (according to alphabetic listings determined by random selection procedures), followed by telephone contact. Post hoc statistical analyses demonstrated that different recruitment methods did not result in different sample characteristics.

Analyses are based on data from women married continuously to the same partner. The initial study sample consisted of 728 participants (the refusal rate was 10.9%); 621 of these were married. Attrition between the initial and fifth follow-up years among married respondents was 12% (547 women). We excluded 117 who changed marital status, 61 who changed study group status (primarily switching from not planning sterilization to undergoing sterilization), 26 who underwent a hysterectomy, and 2 who did not respond to sexuality questions. The resultant sample consisted of 341 women: 152 women in the tubal sterilization group, 106 in the vasectomy wives group, and 83 in the not-planning-sterilization group.

Interview procedures. Baseline information (initial interview) was collected from tubal sterilization women and vasectomy wives immediately before sterilization and from women not planning sterilization soon after recruitment. The interview preceded sterilization by several months for a small minority of women who postponed the procedure. Follow-up interviews were conducted for 5 years at yearly intervals from the first interview date (December 1980 to March 1982). All women were interviewed in locations of their choosing during the initial year and the first 2 years of follow-up. The remaining interviews were conducted by telephone. A reliability check demonstrated that only 4% (13 of 350) of repeat menstrual and psychosocial responses, obtained several days to 1 month after the initial interview, differed from the original.

Measures of sexuality. Mean satisfaction with sexual relationship with husband was measured on a 7-point scale from very low (1) to very high (7). Satisfaction with one's own sexual response was measured on a 4-point scale ranging from not satisfied (1) to very satisfied (4). Average annual frequency of coital desire and coital frequency were obtained by asking women to choose one of the following responses: several times a day (converted to 730 episodes), every day (converted to 365), a few times a week (converted to 182), once a week (converted to 52), a few times a month (converted to 26), once a month (converted to 12), a few times a year (converted to 6), and never (converted to 0). Respondents were more comfortable and thus possibly more forthright in their reporting, selecting from response options than providing an "exact" number. Our nonresponse rate, $<1\%$, was much lower than the 5.3% to 8.3% reported in the National Fertility Studies.[19] We chose an average over the past 12 months as opposed to a shorter interval, e.g., the previous month, because coital frequency often varies. Moreover, since a consid-

erable minority of subjects were 9 months pregnant when interviewed initially, their current data would not have been meaningful. Pregnant women were asked to provide their average frequency before pregnancy. Our aim was not to produce precise frequencies but to examine change through time. Consequently, whereas our annual tallies may not be entirely accurate, there is no reason to question the direction or magnitude of change.

Data analysis. Since a wide variety of factors may influence sexuality, we first examined a correlation matrix associating the four dependent variables at baseline with approximately 70 independent variables, drawn from the following areas: (1) demographic characteristics of respondents and spouses (e.g., age, educational level, Duncan's Socioeconomic Status (SES) scale ratings,[20] religion, employment status, income, and length of marriage); (2) feelings about oneself (e.g., self-confidence, satisfaction with femininity, hopefulness); (3) feelings about one's marriage (e.g., conflict level with spouse, satisfaction with marriage, stability of marriage); (4) pregnancy status; (5) type of contraception used; (6) overall physical health; (7) desire for more children; and (8) Spielberger Trait Anxiety scores.[21] All variables that were significantly related to any of the dependent measures were tested for differences among groups with χ^2 or analyses of variance. The Student–Newman-Keuls test was used to determine which groups differed for those variables for which an overall significant effect was found. Our objective was to identify independent variables that might account for group differences in sexuality. Consequently, only those variables that were significantly or close to significantly ($p < 0.1$) different among groups were included in stepwise (backward and forward) multiple regression analyses with the four sexuality measures as dependent variables.

To simplify and improve the explanatory power of our models and avoid potential problems of colinearity among predictor variables, related items were combined into factors (α-factor extraction, no rotation) for possible use as covariates. Factor scores for each case were computed with the use of factor-score coefficients for each variable, and group differences were ascertained with analysis of variance. We chose as covariates the set of factors and individual variables that differed between groups ($p \leq 0.05$) and accounted for the largest amount of variance in the dependent variable.

The selected covariates were used in a repeated-measures analysis of covariance to determine cross-sectional group differences, i.e., in baseline and overall average (average group effect, considered over all time points) values for each dependent variable. Adjusted means were computed from the inverse of the transformation matrix used in the repeated-measures analysis of covariance. An overall time effect and group differences in rates of change (comparison of slopes) were determined with the use of repeated-measures analysis of variance over the six time points. Changes between pairs of succeeding years and between baseline and each follow-up year were determined with the use of analysis of variance. Overall net change, i.e., the difference between baseline and follow-up year 5 values, was determined through analysis of covariance, with the use of baseline values for pertinent sociodemographic covariates. Power of these analyses ranged between 0.81 and 0.89.

Results

Sociodemographic and psychosocial characteristics of sample. Several sociodemographic differences were noted among the three groups (Table I). Women not planning sterilization were the oldest, were married the longest, were the best educated, and had the highest Duncan's SES scale rating. Their husbands were also the oldest and the best educated. Husbands of vasectomy wives had the lowest income; the combined household income of this group was also the lowest.

All three groups were similar in trait anxiety levels and in the following psychosocial characteristics: evaluation of general happiness, number of personal problems, evaluation of one's own mental health, self-confidence, satisfaction with one's physical attractiveness, satisfaction with division of labor at home, conflict level with husband, effectiveness of marital conflict resolution, evaluation of which spouse acquiesces more in conflict situations, interest in another baby, and involvement with religious activities. Vasectomy wives, however, were most hopeful about the future ($p < 0.02$) and most certain of the stability of their marriage ($p = 0.01$); tubal sterilization women were least satisfied with their role as mother ($p = 0.01$). Differences in satisfaction with marriage and with femininity approached statistical significance, with vasectomy wives being most positive. The two psychosocial factors incorporating these variables (see Table II) varied significantly among groups, with vasectomy wives more positive than tubal sterilization women. All sociodemographic and psychosocial variables were examined for potentially confounding effects on the dependent variables and were controlled for when indicated.

Independent variables related to sexuality. Various age-time, sociodemographic, and psychosocial variables were significantly associated with one or more of the sexuality variables. In general, age, marital duration, educational level, Duncan's SES scale rating, income, and trait anxiety had negative associations. The following psychosocial variables were associated with higher levels of sexual satisfaction and/or activity: positive evaluation of general happiness, hopefulness regarding the future, positive evaluation of one's own mental

Table I. Sociodemographic characteristics of sample at baseline interview (all values are mean ± SE*)

	Tubal sterilization women (n = 152)	*Vasectomy wives (n = 106)*	*Women not planning sterilization (n = 83)*	*p Value†*
Parity (includes current pregnancy)	2.30 ± 0.08	2.19 ± 0.08	2.22 ± 0.08	NS
Years married	8.93 ± 0.44	7.88 ± 0.47	11.03 ± 0.63	0.000‡
Age (yr)	30.77 ± 0.42	30.02 ± 0.51	33.89 ± 0.63	0.000‡
Age of husband (yr)	33.45 ± 0.55	32.28 ± 0.55	36.19 ± 0.74	0.000‡
Duncan's SES scale (if not working, rating of prior work is used)§	52.00 ± 1.54	50.48 ± 1.69	59.12 ± 1.44	0.000‡
Duncan's SES scale, husband	53.50 ± 1.87	56.24 ± 2.10	65.51 ± 2.44	0.001‡
Income (part-time or full-time, dollars)‖	12,932 ± 1674	11,802 ± 1753	13,179 ± 2252	NS
Income, husband¶	$27,992 ± $2718	$20,889 ± $953	$31,533 ± $2598	0.01#
Household income	$33,253 ± $2.73	$25,449 ± $1.27	$36,556 ± $2.87	0.01#
Education (yr)	13.61 ± 0.17	13.97 ± 0.20	15.05 ± 0.28	0.000‡
Education of husband (yr)	14.54 ± 0.22	14.84 ± 0.26	16.41 ± 0.38	0.000‡
Religious affiliation (%)				NS
Protestant	62.5	55.7	49.4	
Catholic	19.1	28.3	27.7	
Other (fundamentalist, Mormon, Jewish, none)	18.4	16.0	22.9	
Employed (%)	42.8	39.6	42.2	NS

*Standard error not relevant for proportional data.

†*p* Values are based on the F statistic for means and the χ^2 statistic for differences among proportions where percentages are provided.

‡Women not planning sterilization are different.

§Sample sizes are 149, 104, and 82.

‖Sample sizes are 65, 41, and 35.

¶Sample sizes are 148, 104, and 78.

#Vasectomy wives are different.

Table II. Independent variables including factors* used as covariates (mean values and direction of effect) in the analyses of various sexuality variables

	Direction of effect	*Tubal sterilization women (n = 152)*	*Vasectomy wives (n = 106)*	*Women not planning sterilization (n = 83)*	*p Value (group differences)*
Education (yr)	Negative	13.61	13.97	15.05	0.000†
Years married	Negative	8.93	7.88	11.03	0.000†
Age-time factor‡	Negative	−0.064	−0.226	0.405	0.000†
SES factor§	Negative	−0.115	−0.108	0.348	0.000†
Psychosocial factor 1‖	Positive	−0.149	0.199	0.018	0.002¶
Psychosocial factor 2#	Positive	−0.090	0.123	0.008	0.033¶

*Standardized factor scores have a mean of 0 and a standard deviation of 1.

†Women not planning sterilization are different.

‡This factor consists of the following variables: woman's age and length of marriage.

§This factor consists of the following variables: woman's Duncan's SES scale rating and husband's education.

‖This factor consists of the following variables: satistaction with femininity, satisfaction with marriage, certain marriage will remain stable and satisfying, and satisfaction with maternal role.

¶Tubal sterilization women are different from vasectomy wives.

#This factor consists of the following variables: satisfaction with femininity and satisfaction with marriage.

health, self-confidence, satisfaction with physical attractiveness, satisfaction with femininity, satisfaction with marriage, happy with the division of labor at home, low overall conflict with husband, effective conflict resolution with husband, feeling that marriage will remain stable and satisfying in the future, and satisfaction with maternal role. Group means for all independent variables, including factors, chosen as covariates appear in Table II. The remaining tables indicate which covariates were used in the analysis of each sexuality variable.

Change in sexuality through time

Satisfaction with one's sexual relationship. As shown in Table III, there were no significant group differences in adjusted values for satisfaction with one's sexual relationship at baseline or averaged (average group ef-

Table III. Mean* satisfaction with sexual relationship† among tubal sterilization women (n = 152), vasectomy wives (n = 106), and women not planning sterilization (n = 83)

	Tubal sterilization women		*Vasectomy wives*		*Women not planning sterilization*		*p Value (group differences‡)*
	Observed	*Adjusted*	*Observed*	*Adjusted*	*Observed*	*Adjusted*	
Baseline year	5.72	5.79	5.82	5.68	5.68	5.75	NS
First follow-up year	5.61	5.68	5.75	5.61	5.28	5.35	NS
Second follow-up year	5.50	5.57	5.75	5.61	5.33	5.40	NS
Third follow-up year	5.48	5.55	5.78	5.64	5.31	5.38	NS
Fourth follow-up year	5.56	5.63	5.82	5.68	5.38	5.45	NS
Fifth follow-up year	5.41	5.48	5.56	5.42	5.29	5.36	NS
Net change	−0.31		−0.26		−0.39		NS
Overall average group effect p = NS							
Overall time effect p = 0.000							
Group differences in rate of change over time p = NS							

*Means are presented as both observed and adjusted (for effects of significant covariates) values. Covariates include woman's education, years married, and psychosocial factor 1 (R^2 = 0.321, p = 0.000). p Values are based on the F statistic for means.

†Scale ranges from 1 (very low) to 7 (very high).

‡Refers to group differences in baseline values, in changes between succeeding years, or in changes between baseline and each follow-up year. In cases of significant differences the specific comparison is indicated in a footnote.

fect) over the 5-year period (without adjustments, vasectomy wives were significantly [p = 0.02] more satisfied over 5 years than were women not planning sterilization). There was, however, an overall time effect: all groups decreased significantly (an average of 0.31 or 5.5%), moving from the category "high" toward "mostly high." There were no significant group differences in rate of change, overall net change, or changes between any two given years.

Satisfaction with one's own sexual response. As shown in Table IV, satisfaction with one's own sexual response did not change significantly through time. With or without covariates, there was no significant group effect on mean values; throughout the study all groups remained between moderately and very satisfied (but closer to the moderate category) with their sexual response. There were no significant group differences in rate of change, overall net change, or changes between any two given years.

Frequency of coital desire. There were no significant group differences (see Table V) in adjusted values for coital desire at baseline or averaged over the 5-year period (without adjustments, women not planning sterilization had significantly [$p < 0.04$] lower average frequencies than did either sterilization group). There was, however, an overall time effect: all groups had a significant decrease (an average of 15.3 coital episodes or 11.5%). There were no group differences in rate of change or in overall net change. Duration of marriage was negatively related ($p < 0.02$) to net decrease in desire: Women married the shortest time at baseline showed the largest declines during the 5-year period.

Although there were no group differences in overall net change, there was a difference in pattern of change. Follow-up values for both sterilization groups were higher than initial values until the fifth follow-up year, at which time they dropped below baseline levels. Among tubal sterilization women, coital desire peaked at the first follow-up year. However, the relatively large increase was not significantly different from the slight increase shown by vasectomy wives or the slight decrease shown by women not planning sterilization. Wives of vasectomized men showed a steady increase in desire, peaking at follow-up year 3. Women not planning sterilization, on the other hand, exhibited an immediate decline, with the largest drop at follow-up year 4. At this time the net change experienced by both sterilization groups (an increase) was significantly (p = 0.03) different from that of women not planning sterilization (a decrease). However, by follow-up year 5, when all groups showed a decline, the overall net change, although larger for women not planning sterilization, was not significantly different.

Coital frequency. There were no significant group differences (see Table VI) in adjusted values for coital frequency at baseline or averaged over the 5-year period (without adjustments, women not planning sterilization had significantly lower [$p < 0.03$] average frequencies than did either sterilization group). The difference made by covariates is seen clearly in Fig. 1, where observed and adjusted coital frequency values are compared graphically. Without covariates there is a clear difference between the sterilization groups and women not planning sterilization. With covariates, the time lines are close to one another.

There was an overall effect of time: All groups had a significant decline (an average of 19.7 episodes or 14.4%); there were no group differences in rate of

Table IV. Mean* satisfaction with own sexual response† among tubal sterilization women (n = 152), vasectomy wives (n = 106), and women not planning sterilization (n = 83)

	Tubal sterilization women		*Vasectomy wives*		*Women not planning sterilization*		*p Value (group differences‡)*
	Observed	*Adjusted*	*Observed*	*Adjusted*	*Observed*	*Adjusted*	
Baseline year	3.29	3.30	3.31	3.27	3.18	3.21	NS
First follow-up year	3.24	3.25	3.31	3.27	3.04	3.07	NS
Second follow-up year	3.26	3.27	3.40	3.36	3.15	3.18	NS
Third follow-up year	3.22	3.23	3.32	3.28	3.07	3.10	NS
Fourth follow-up year	3.27	3.28	3.26	3.21	3.16	3.19	NS
Fifth follow-up year	3.22	3.23	3.30	3.26	3.13	3.16	NS
Net change	−0.07		−0.01		−0.05		NS
Overall average group effect p = NS							
Overall time effect p = NS							
Group differences in rate of change over time p = NS							

*Means are presented as both observed and adjusted (for effects of significant covariates) values. Covariates include woman's education and psychosocial factor 2 (R^2 = 0.124, p = 0.000). p Values are based on the F statistic for means.

†Scale ranges from 1 (not satisfied) to 4 (very satisfied).

‡Refers to group differences in baseline values, in changes between succeeding years, or in changes between baseline and each follow-up year. In cases of significant differences the specific comparison is indicated in a footnote.

Table V. Mean* annual frequency of coital desire† among tubal sterilization women (n = 152), vasectomy wives (n = 106), and women not planning sterilization (n = 83)

	Tubal sterilization women		*Vasectomy wives*		*Women not planning sterilization*		*p Value (group differences‡)*
	Observed	*Adjusted*	*Observed*	*Adjusted*	*Observed*	*Adjusted*	
Baseline year	134.6	133.3	136.9	130.1	128.9	136.9	NS
First follow-up year	153.2	151.9	140.6	133.8	125.5	133.5	NS
Second follow-up year	140.7	139.4	143.4	136.6	116.9	125.0	NS
Third follow-up year	140.1	138.8	150.5	143.7	117.3	125.4	NS
Fourth follow-up year	137.2	135.9	145.1	138.3	100.8	108.9	0.03§
Fifth follow-up year	122.1	120.8	128.3	121.5	99.6	107.7	NS
Net change	−12.5		−8.6		−29.3		NS
Overall average group effect p = NS							
Overall time effect p = 0.000							
Group differences in rate of change over time p = NS							

*Means are presented as both observed and adjusted (for effects of significant covariates) values. Covariates include woman's education, years married, and psychosocial factor 2 (R^2 = 0.134, p = 0.000). p Values are based on the F statistic for means.

†Scale ranges from never to several times a day.

‡Refers to group differences in baseline values, in changes between succeeding years, or in changes between baseline and each follow-up year. In cases of signficant differences the specific comparison is indicated in a footnote.

§Changes (between baseline and fourth follow-up year) experienced by tubal sterilization women and vasectomy wives (increases) are different from change experienced by women not planning sterilization (decrease).

change or in overall net change. By follow-up year 5, women who had undergone tubal sterilization showed the smallest (although not significantly different) net decline. As in the case of coital desire, duration of marriage had a significant impact on net decline, with women married the shortest time showing the largest drop ($p < 0.01$).

Although there were no long-term group differences, tubal sterilization women showed a significant increase in coital frequency at first-year follow-up, compared with both vasectomy wives (slight decrease) and women not planning sterilization (large decrease). The following year they experienced a significant decrease, bringing them back in line with the rest of the sample. After this point their pattern was similar to that of the other groups, i.e., an overall decline.

Despite their increase in both coital desire (not significant) and coital frequency, tubal sterilization women were not initiating intercourse more frequently than they had during the preceding year; nor were there changes in husband-wife patterns regarding which partner desired intercourse more often. In both years

Table VI. Mean* annual coital frequency† among tubal sterilization women (n = 152), vasectomy wives (n = 106), and women not planning sterilization (n = 83)

	Tubal sterilization women		*Vasectomy wives*		*Women not planning sterilization*		*p Value (group differences‡)*
	Observed	*Adjusted*	*Observed*	*Adjusted*	*Observed*	*Adjusted*	
Baseline year	136.7	135.5	148.0	141.1	129.3	137.5	NS
First follow-up year	152.7	151.5	143.5	136.6	114.1	122.2	0.002§
Second follow-up year	132.7	131.5	144.9	137.9	114.9	123.0	0.01‖
Third follow-up year	129.6	128.5	142.0	135.1	118.2	126.4	NS
Fourth follow-up year	127.5	126.3	137.0	130.1	107.1	115.2	NS
Fifth follow-up year	124.0	122.8	124.2	117.3	102.0	110.1	NS
Net change	−12.7		−23.8		−27.3		NS

Overall average group effect p = NS
Overall time effect p = 0.000
Group differences in rate of change over time p = NS

*Means are presented as both observed and adjusted (for effects of significant covariates) values. Covariates include psychosocial factor 2, age-time factor, and SES factor (R^2 = 0.118, p = 0.000). p Values are based on the F statistic for means.

†Scale ranges from never to several times a day.

‡Refers to group differences in baseline values, in changes between succeeding years, or in changes between baseline and each follow-up year. In cases of significant differences, the specific comparison is indicated in a footnote.

§Change (between baseline and first-year follow-up) experienced by tubal sterilization women (increase) is different from that experienced by women not planning sterilization and vasectomy wives (decreases).

‖Change (between first and second follow-up years) experienced by tubal sterilization women (decrease) is different from that experienced by women not planning sterilization and vasectomy wives (slight increases).

tubal sterilization women perceived that sexual activity either was initiated equally by both partners (53% in both years) or was always or usually initiated by the husband (43% at baseline, 44% at follow-up year 1). Again, in both years they perceived either that there were no spousal differences in coital desire (40% and 42%) or that their husbands desired it more often (53% and 50%). There were no group differences in either of these variables with respect to mean values or change through time.

The experience of a coital frequency increase by tubal sterilization women and not by vasectomy wives was not readily interpretable. At first-year follow-up respondents in both groups were asked if they had feared pregnancy before sterilization and how they currently felt. Approximately 45% of tubal sterilization women and 54% of vasectomy wives initially feared pregnancy; 96% of the former group and 91% of the latter group were no longer afraid. There were no group differences either in initial fear of pregnancy or in the subsequent relief of that fear. Given the absence of a straightforward explanation, we are conducting further analyses, which will be published later.

Comment

Examination of sexual change during a 5-year period indicated (1) overall declines in satisfaction with sexual relationship, in coital desire, and in coital frequency; (2) confounding negative effects of age, marital duration, income, education, and occupational SES and positive effects of psychosocial factors (related to satisfaction with marriage and oneself) on cross-sectional measures of sexuality; and (3) an inverse relationship between marital duration and overall net decline in coital desire and frequency. These findings are consistent with those reported in the literature.

Decrease in marital coital frequency through time is well documented. Although some controversy exists, it appears to be primarily a function of marital duration, age of the male partner, and/or age of the female partner.[19, 22-26] Whereas coital frequency decreases as marital duration increases (probably because of reduced novelty, decreased energy levels, and increased time commitments to children and career), declines for individuals studied at different time points indicate the sharpest drops occur in the earlier years of marriage, presumably when reduced novelty has its greatest impact.[23, 24] The positive relationship between sexuality and general marital adjustment and the dampening effect of higher educational attainment on couple sexuality have also been previously reported.[16, 23, 26] The former relationship makes good theoretical sense but the latter requires some explanation. It is theorized that couples with higher incomes (and presumably higher educational attainment) have greater access to leisure-time alternatives to sexual activity.[23] They may also be employed in more demanding, high-stress positions and thus have less leisure time and less physical and psychic energy for sexual activity.

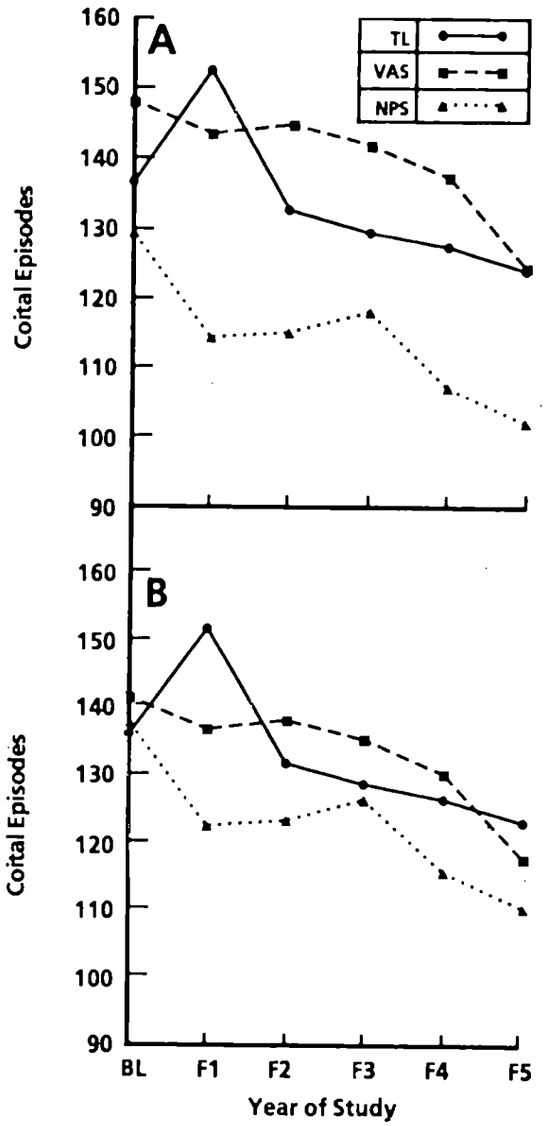


Fig. 1. A, Observed number of annual coital episodes among tubal sterilization women (*TL*, $n = 152$), vasectomy wives (*VAS*, $n = 106$), and women not planning sterilization (*NPS*, $n = 83$). **B,** Adjusted number of annual coital episodes among tubal sterilization women (*TL*, $n = 152$), vasectomy wives (*VAS*, $n = 106$), and women not planning sterilization (*NPS*, $n = 83$).

Change in satisfaction with one's own sexual response has not been previously addressed. However, its remarkable stability over time makes sense in that it reflects an internal state and is less influenced by external circumstances.

Our major concern was the long-term impact of male and female sterilization on female marital sexuality. Our data demonstrate an overall absence of group differences in four dimensions of sexuality considered over a 5-year period. No long-term effects of either type of sterilization were noted. Group differences in observed average values were a function of differences in sociodemographic and psychosocial characteristics. Once these effects were removed, group differences disappeared.

The two short-term group differences noted were a decrease in coital desire among women not planning sterilization between baseline and the fourth follow-up year, compared with increases experienced by both sterilization groups and an increase in coital frequency at the first follow-up year only in tubal sterilization women. The latter change is similar to that observed by Cooper et al.[18]

The increase in coital desire among sterilization groups appears related to the sterilization procedures and the freedom from fear of pregnancy they confer. These data suggest that sterilization may act as a short-term buffer against the natural tendency for aging and increasing marital duration to depress sexual desire.

The explanation for the temporary coital frequency increase in the tubal sterilization group alone is less apparent. Freedom from pregnancy fears, while certainly related to increased coital frequency, does not account for this difference. Tubal sterilization women were not more fearful of pregnancy than were vasectomy wives; nor were they more often relieved afterward. We are conducting detailed analyses to investigate this phenomenon. In the interim we speculate that taking charge of one's *own* reproduction may elicit positive feelings about oneself and foster the ultimate purpose of non–medically indicated sterilization—worry-free coital activity. Moreover, a woman who takes it on herself to be sterilized may want (consciously or not) to realize immediate benefit.

However, increased coital activity was not effected by more frequent female initiation of sexual activity or by a change in husband-wife patterns regarding which partner desired intercourse more often. Overall, as perceived by women, men remained more desirous and the more usual initiators of sexual intercourse. Perhaps women who had undergone tubal sterilization became more receptive to coital activity than they had been in the past and somehow communicated their heightened desire, a behavior they may not have considered outright initiation.

These data provide some assurance, within the limitations of our sample, regarding the short- and long-term sexual consequences of non–medically indicated tubal sterilization and vasectomy. There appear to be no detrimental effects and some short-term benefits of both procedures on female marital sexuality.

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The "Allis" test for easy cesarean delivery

Michael A. Finan, MD, Dimitrios S. Mastrogiannis, MD, PhD, and William N. Spellacy, MD
Tampa, Florida

Cesarean delivery may be complicated by dystocia if the incision size is inadequate. A test is described that may be easily applied at cesarean section, creating greater objectivity in the determination of surgical incision size. One hundred patients were studied prospectively. Patients with incisions that passed the test had a shorter mean time of delivery and less difficult deliveries than those of patients with incisions that failed the test. We conclude that the "Allis" test is a simple way to reduce the incidence of dystocia at cesarean delivery. (AM J OBSTET GYNECOL 1991;164:772-5.)

Key words: Test, cesarean section, dystocia

Cesarean section is the most common major surgical procedure in the field of obstetrics and gynecology.[1] Cosmetic considerations may exert pressure on the surgeon to produce incisions that are small, and thus less disfiguring. However, smaller incisions often restrict exposure of the operative field and may increase the difficulty of delivery.

In 1987 Ayers and Morley[2] determined that an abdominal incision size ≥15 cm is associated with significantly less difficulty in cesarean delivery. Their efforts stimulated interest in the development of an efficient and simple way to test the wound size and thus reduce the incidence of difficulty at cesarean delivery. The purpose of this study was to develop a test that would predict easy cesarean delivery and thus reduce dystocia and speed delivery at cesarean section.

The test proposed is to use a common instrument, the Allis forceps, as a wound size gauge. This forcep measures approximately 15 cm in length and is readily available.

Material and methods

Between Jan. 1 and April 30, 1990, 100 patients undergoing cesarean section at Tampa General Hospital were prospectively entered in the study.

The surgery was performed by the resident staff and the attending physicians. A vertical or Pfannenstiel skin incision was performed. After entering the peritoneal cavity, two Richardson retractors were used to retract the abdominal walls laterally. An Allis clamp was then held between the retractors. The test was described as "passed" if the clamp easily fit between the retractors and "failed" if the clamp was longer than the distance between the retractors, as in Fig. 1. The cesarean delivery was undertaken in the usual fashion,[3,4] regardless of the outcome of the test. All the uterine incisions were of the low-transverse type. To objectively assess difficulty of delivery a circulating nurse recorded the length of time in seconds between the start of the uterine incision, after the bladder was removed from the lower segment, and delivery of the fetus. The operators subjectively graded the difficulty of delivery with a scale of I to IV as follows: I = no dystocia, very easy delivery; II = moderate effort required to effect delivery; III = forceful abdominal pressure required for delivery; IV = extension of the incision or muscle splitting was required for delivery.

Table I. Operative and delivery data

	Passed group		*Failed group*		
	No.	*%*	*No.*	*%*	*p Value*
Skin incision					
Pfannenstiel	31	79	54	88	NS
Vertical	8	21	7	12	NS
Anesthesia					
Epidural	22	56	49	80	NS
General	10	26	5	8	NS
Spinal	7	18	7	12	NS
Presentation					
Vertex	24	61	44	72	NS
Breech	15	39	17	28	NS
Sex					
Male	22	56	36	59	NS
Female	17	44	25	41	NS
Cesarean section					
Primary	28	72	48	79	NS
Repeat	11	28	13	21	NS

From the Department of Obstetrics and Gynecology, University of South Florida College of Medicine.
Received for publication June 22, 1990; revised October 22, 1990; accepted November 6, 1990.
Reprint requests: Michael A. Finan, MD, Department of Obstetrics and Gynecology, Suite 529, Harbour-Side Medical Tower, 4 Columbia Dr., Davis Islands, Tampa, FL 33606.
6/1/26585

Table II. Demographic and delivery data

	Passed group		*Failed group*		
	Mean	*Range*	*Mean*	*Range*	*p Value*
Age (yr)	25.3	16-35	22.7	16-35	0.02
Parity	1.2	0-5	0.7	0-6	0.03
Gestational age (wk)	38.8	28-42	39.1	34-43	NS
Birth weight (gm)	3505	1100-5585	3305	1950-4850	NS
Cord pH	7.26	7.07-7.36	7.26	7.15-7.34	NS
Time from incision to delivery (seconds)	58.4	15-190	95.7	30-270	0.002

Obstetric and epidemiologic data were obtained from the medical records of these patients. The results were analyzed statistically with the Student's t test and χ^2 test where appropriate. The analysis was performed with the assistance of the SPSS statistical computer package. A p value of <0.05 was considered significant.

Results

Of the 100 patients entered in the study, 39 incisions passed the "Allis" test and 61 incisions failed the test. The patients were similar with regard to type of skin incision, type of anesthesia, presentation, and sex of the fetus, as shown in Table I. Sixty-eight (68%) of the patients had a fetus in the vertex presentation and 32 (32%) were breech. In the group with incisions that passed the test, 24 (61%) were vertex and 15 (39%) were breech. In the group with incisions that failed the test, 44 (72%) were vertex and 17 (28%) were breech. These differences were not statistically significant. A total of 85% of the skin incisions were Pfannenstiel and 15% were vertical. The majority of cesarean sections were primary (76%) with 24% being repeat cesarean sections. Of the group with incisions that passed the test, 28 (72%) underwent primary and 11 (28%) underwent repeat cesarean sections. Of the group with incisions that failed the test, 48 (79%) were primary and 13 (21%) were repeat cesarean sections. These differences were not statistically significant.

The group with incisions that passed the "Allis" test had a higher mean age and parity than those of the group with incisions that failed the test. The groups were similar with regard to gestational age, birth weight, and cord pH as shown in Table II.

The patients with incisions that passed the test had a shorter length of time from uterine incision to delivery than that of those with incisions that failed the test. The mean time of delivery was 58.4 seconds (range, 15 to 190) for those with incisions that passed the test and 95.7 seconds (range, 30 to 270) for those with incisions that failed the test ($p < 0.005$).

The group with incisions that passed the test had a statistically significant difference ($p < 0.05$) between the 1-minute Apgar scores for each grade of dystocia. In the group with incisions that failed the test, we found no difference between the 1-minute Apgar scores and the four grades of dystocia (see Table III). Comparison of the Apgar scores for every grade of dystocia within each of the two groups (passed and failed) did not show a statistically significant difference.

Fig. 2 shows the percentage of patients in each group (passed versus failed) with a particular grade of dystocia. A total of 26.3% of patients with incisions that failed the test had a grade III or IV delivery, whereas

Table III. Relationship of grade of dystocia to 1-minute Apgar scores

Grade of dystocia	*1 min Apgar score*	*Passed group*		*Failed group*		*p Value*
		No.	*%**	*No.*	*%†*	
I	9	19	48.7	13	21.4	NS
	8	4	10.2	3	4.9	NS
	7	—	—	1	1.6	NS
	6	4	10.2	1	1.6	NS
	5	1	2.6	2	3.3	NS
	4	—	—	1	1.6	NS
	3	—	—	—	—	—
II	9	4	10.2	14	22.9	NS
	8	1	2.6	6	9.9	NS
	7	1	2.6	2	3.3	NS
	6	2	5.1	1	1.6	NS
	5	—	—	2	3.3	NS
III	9	1	2.6	7	11.6	NS
	8	—	—	2	3.3	NS
	7	—	—	1	1.6	NS
	6	1	2.6	1	1.6	NS
IV	9	—	—	3	4.9	NS
	8	—	—	—	—	—
	7	—	—	—	—	—
	6	—	—	—	—	—
	5	1	2.6	—	—	NS
	4	—	—	—	—	—
	3	—	—	1	1.6	NS

*p Value, 0.014.
†p Value, NS.

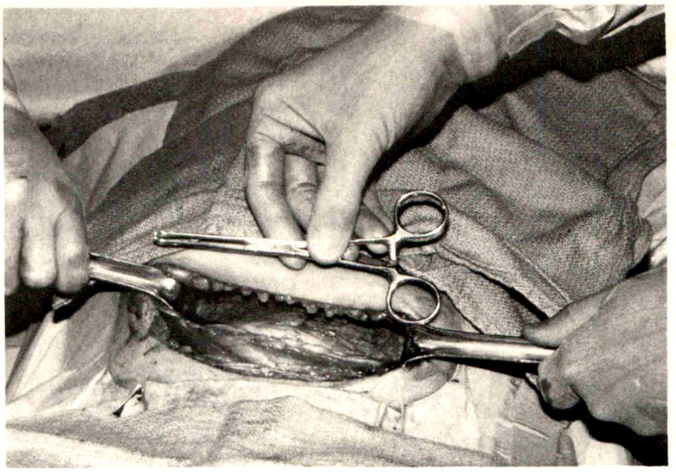


Fig. 1. Use of Allis test quickly shows that incision may be too small to allow easy delivery.

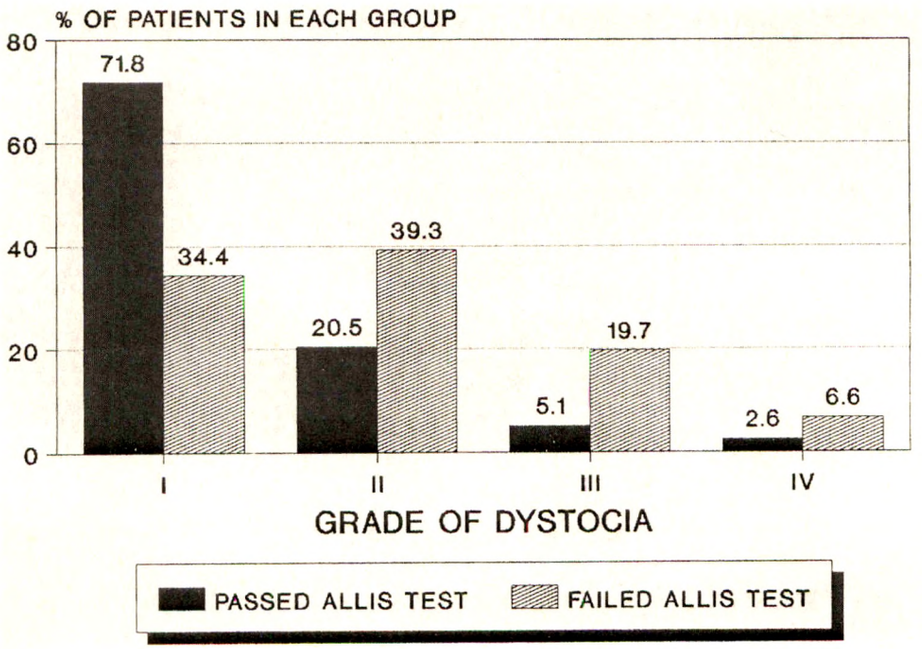


Fig. 2. Percentage of patients in each group for each given grade of dystocia.

only 7.7% of those with incisions that passed the test had a delivery of this difficulty ($p < 0.05$).

Comment

The tendency of surgeons to create smaller incisions for cosmetic reasons is understandable. *Williams' Obstetrics*[4] states that the cesarean section incision should be of sufficient length to permit delivery of the infant without difficulty. Ayers and Morley[2] determined that difficult deliveries, in which forceful pressure on the maternal abdomen was required to effect delivery or in which enlargement of the incision was required for delivery of the infant, were related to an incision size <15 cm. The same study suggested that the degree of difficulty during delivery of the fetus correlates inversely with incision size.

The present study evaluated prospectively a test that would predict easier, more rapid delivery at cesarean section. The groups were similar with regard to birth weight, gestational age, and cord pH. The fact that the group with incisions that passed the "Allis" test had a higher mean age and parity than those of the group

with incisions that failed the test could be explained because of more relaxed abdominal walls in this group.[5]

The group with incisions that passed the "Allis" test who were delivered of infants with grades I and II dystocia had significantly higher 1-minute Apgar scores than those with grades III and IV dystocia ($p < 0.05$). This difference was not found for the group with incisions that failed the test. Comparison of 1-minute Apgar scores of the two groups (passed versus failed) shows there was no statistically significant difference. There was also no difference within each group or between the groups in the 5-minute Apgar scores. These differences may be elicited with larger numbers in each group.

The failed "Allis" test was associated with a longer delivery time and a significantly more difficult delivery than was the passed "Allis" test.

Factors that affect difficulty of cesarean delivery are varied. They include parity, abdominal wall relaxation, station, position of the presenting part, and size of surgical incision. One of the factors that we can control to reduce the incidence of dystocia at cesarean delivery is the size of the surgical incision. We conclude that application of the "Allis" test is an objective way to control the size of surgical incision at cesarean delivery.

The Allis clamp measures approximately 15 cm and is readily available. If the instrument fits between two retractors that distend the abdominal incision laterally, the delivery can be carried out easily.

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Flow velocity waveforms of the fetal circulation preceding fetal death in a case of lupus anticoagulant

Giancarlo Mari, MD,[a] and Nathan Wasserstrum, PhD, MD[a, b]
Houston, Texas

Flow velocity waveforms of the vascular system were evaluated at 25 weeks 2 days and 27 weeks 5 days of gestation in the case of a severely growth-retarded fetus. At the first scan, the cerebral vessels displayed low pulsatility indexes, reflecting the brain-sparing pattern that characterizes fetal stress. This compensatory reduction was lost at the time of the second, "preterminal" scan. (AM J OBSTET GYNECOL 1991;164:776-8.)

Key words: Brain-sparing effect, fetal Doppler, intrauterine growth retardation, pulsatility index

Flow velocity waveforms of the umbilical artery, middle cerebral artery, and other vessels have been described in normal fetuses. Characteristic changes in the waveforms have been reported in association with chronic fetal stress and growth retardation.[1] The changes that the latter pattern may undergo as a reflection of preterminal decompensation in the last hours or days before intrauterine death have not yet been defined. We assessed the Doppler flow velocity waveforms of various fetal arteries, the ductus arteriosus, and the semilunar valves in a growth-retarded fetus. The pregnancy was complicated by antiphospholipid syndrome (lupus anticoagulant). Doppler studies were performed at 25 weeks 2 days and again at 27 weeks 5 days of gestation. The fetus died in utero within 24 hours of the second scan. Hence we were able to characterize the various Doppler flow velocity waveforms within 24 hours of fetal death.

Case report

Routine first-trimester laboratory evaluation of a 32-year-old Oriental woman, gravida 1, para 0, revealed a positive reactive plasma reagin test. Fluorescent treponemal antibody test results were negative. Pertinent results of further evaluation included an elevated partial thromboplastin time of 51 seconds, a normal prothrombin time of 11.5 seconds, a positive lupus anticoagulant battery, anticardiolipin antibodies (enzyme-linked immunosorbent assay) that were positive for immunoglobulin G (>6 multiples of the mean) and strongly positive for immunoglobulin M (>12 multiples of the mean), and an anti–deoxyribonucleic acid antibody titer >1:320. There was no history of thrombotic events or systemic lupus erythematosus.

At 17 weeks' gestational age, a diagnosis of antiphospholipid syndrome (lupus anticoagulant) was made and the patient was started on a regimen of low-dose aspirin (80 mg twice per day). Ultrasonographic measurements at this time were consistent with menstrual age. A repeat ultrasonogram at 25 weeks yielded an estimated fetal weight of 430 gm, head circumference of 19.5 cm, and abdominal circumference of 14.3 cm. The patient was hospitalized with a diagnosis of fetal growth retardation associated with antiphospholipid syndrome.

Biophysical evaluation revealed normal fetal breathing and body movements and tone. Amniotic fluid volume was subjectively somewhat decreased. Amniocentesis revealed no chromosomal abnormalities. Prednisone (20 mg per day) and supplemental oxygen were added to the patient's regimen.

At 25 weeks 2 days, after informed consent was obtained from the patient and with the consent of her attending obstetricians, we evaluated fetal vascular flow patterns via Doppler ultrasonography. The results were made available to the patient's obstetricians. At 27 weeks 5 days ultrasonography showed no fetal breathing or body movements and moderately decreased amniotic fluid. We performed a repeat Doppler evaluation. The following day, fetal cardiac activity was absent. Labor was induced and a male infant weighing 430 gm, with Apgar scores of 0 at both 1 and 5 minutes, was delivered vaginally.

Doppler studies were performed with a General Electric PASS II dynamic image scanner. The pulsatility index was calculated as the difference of peak systolic velocity minus lowest diastolic velocity, divided by mean velocity. At the first Doppler evaluation, performed at 25 weeks 2 days the pulsatility index values of the fetal middle cerebral artery (Fig. 1), internal carotid artery, and anterior cerebral artery (Table I) were all abnor-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology,[a] and the Department of Medicine,[b] Baylor College of Medicine.
Received for publication June 28, 1990; revised September 6, 1990; accepted September 14, 1990.
Reprint requests: Giancarlo Mari, MD, Department of Obstetrics and Gynecology, Yale University School of Medicine, 123 York St., #17-J, New Haven, CT 06511.
6/1/25748

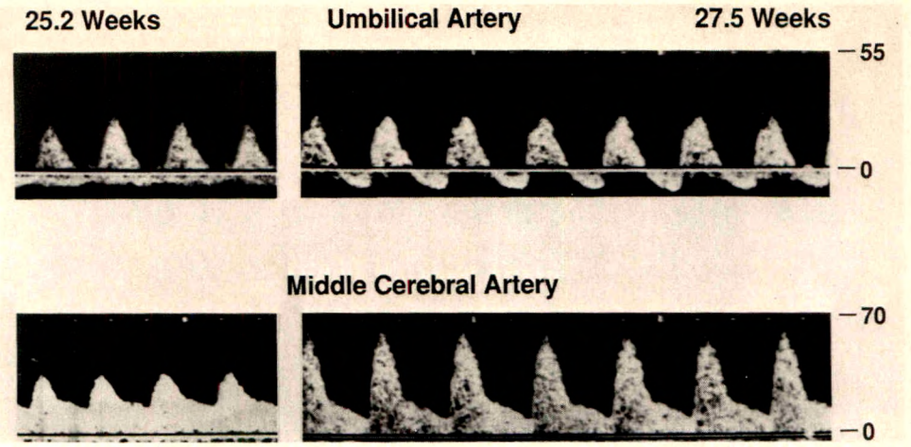


Fig. 1. Flow velocity waveforms of umbilical artery *(top)* and middle cerebral artery *(bottom)*. On left are waveforms from 25 weeks 2 days of gestation. On right are waveforms from 27 weeks 5 days of gestation. Values are in centimeters per second.

Table I. Values obtained with Doppler ultrasonography

Vessel	*25 wk 2 days*	*Normal value (range)*	*27 wk 5 days*	*Normal value (range)*
Pulsatility index				
Middle cerebral artery	0.85	1.5-2.4	1.70	1.6-2.6
Internal carotid artery	0.80	1.2-1.9	1.48	1.3-2.0
Anterior cerebral artery	0.83	1.3-2.2	1.55	1.4-2.3
Umbilical artery	2.20	1.0-1.5	3.50	0.95-1.4
Thoracic aorta	—	—	4.10	—
Abdominal aorta	3.60	—	4.00	—
Renal artery	—	—	4.50	2.0-3.8
Iliac common artery	—	—	3.90	—
Peak systolic velocity				
Ductus arteriosus (cm/sec)	70	—	65	—
Pulmonary valve (cm/sec)	55	—	60	—
Aortic valve (cm/sec)	60	—	60	—
Tricuspid valve	No tricuspid insufficiency		No tricuspid insufficiency	

mally low, reflecting reduced impedance to cerebral blood flow. Diastolic velocity was absent in the umbilical artery and in the abdominal aorta (Table I). There was no evidence of constriction of the ductus arteriosus or associated tricuspid insufficiency. Peak systolic velocities at the pulmonary and aortic valves were normal. Fetal heart rate at the time of Doppler measurements was 160 beats/min.

At the second Doppler evaluation performed at 27 weeks 5 days the pulsatility index values of the middle cerebral artery (Fig. 1), internal carotid artery, and anterior cerebral artery all had risen (Table I) to the normal range, i.e., the range expected in a normal, nonstressed fetus. Diastolic velocity was reversed in the umbilical artery (Fig. 1), the thoracic and abdominal aorta, and the internal iliac artery and was absent in the renal artery (Table I). Peak systolic velocities of the ductus arteriosus, and the pulmonary and aortic valves were unchanged from the preceding examination (Table I). Fetal heart rate was 150 beats/min.

Comment

Severe growth retardation at 25 weeks 2 days of gestation, in the context of the antiphospholipid syndrome, reflects chronic fetal stress. Absence of the Doppler flow velocity waveforms of the umbilical artery and abdominal aorta, further support the diagnosis of fetal hypoxemia. Of interest, routine biophysical index values of fetal status (breathing and body movements and tone) were still normal at this time, despite markedly abnormal Doppler waveforms.

On repeat evaluation 2 weeks later, the umbilical artery and aortic waveforms showed further deterioration, with higher pulsatility index and the appearance of reversed flow. Abnormal diastolic velocity patterns also were noted in vessels not examined at the earlier examination, i.e., the renal and common iliac arteries and the thoracic aorta.

At the time of the first scan the pulsatility index values of all three cerebral arteries were "abnormally" low,

i.e., below values expected in the normal fetus. These "abnormalities" presumably reflected a brain-sparing effect that characterizes chronic fetal hypoxia, i.e., an appropriate compensatory decrease in cerebrovascular impedance and a redistribution of blood flow in favor of the brain. These studies are consistent with studies in the acutely hypoxemic fetal lamb, which have directly demonstrated such a redistribution of flow. Of special note, this brain-sparing effect was no longer present at the time of the second scan, shortly before intrauterine fetal death. We speculate that these changes could indicate extremely severe fetal compromise and a reduction in cerebral blood flow. These results would be in agreement with those observed in fetal sheep, in which a reduction in cerebral blood flow has been observed during sustained hypoxemia and acidemia.[2] Under these circumstances, concomitant reduction of renal blood flow occurs. Loss of brain-sparing effect with persistent absent or reversed diastolic flow of the umbilical artery raises concern about particularly severely compromised fetal status, possibly signaling imminent fetal death.

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Recurrent herpes gestationis with postpartum flare: A case report

Laxmi V. Baxi, MD, Oormila P. Kovilam, MD, Margaret H. Collins, MD, and Robert R. Walther, MD

New York, New York

A case of herpes gestationis recurring in each pregnancy, with a postpartum flare-up in the last pregnancy, is described. The diagnosis was confirmed by skin biopsy. The first pregnancy ended in a term stillbirth, but there were favorable outcomes in four subsequent pregnancies. Histopathologic examination of the placentas of the last three pregnancies revealed varying grades of villositis. Symptoms improved with oral corticosteroids. (AM J OBSTET GYNECOL 1991;164:778-80.)

Key words: Herpes gestationis, autoimmune disorder in pregnancy

Herpes gestationis is a rare skin disorder, which is peculiar to pregnancy and the postpartum period and is characterized by vesiculobullous lesions. This disorder presents a diagnostic challenge and needs close monitoring to reduce maternal and fetal morbidity.

From the Departments of Obstetrics and Gynecology, Pathology, and Dermatology, College of Physicians and Surgeons, Columbia University and Columbia Presbyterian Medical Center.
Received for publication July 6, 1990; revised October 12, 1990; accepted October 18, 1990.
Reprint requests: Laxmi Baxi, MD, College of Physicians and Surgeons, Columbia University, Department of Obstetrics and Gynecology, 622 W. 168th St., New York, NY 10032.
6/1/26203

Case report

In a 31-year-old woman (gravida 6, para 4) progressively increasing pruritus developed on the trunk and extremities beginning in the third trimester. These symptoms and signs responded minimally to oral antihistamines and a topical hydrocortisone cream. In the current pregnancy fetal well-being was assured by nonstress tests starting at 36 weeks' gestation. At 38 weeks the patient was delivered of a female infant weighing 3000 gm with Apgar scores of 9 and 9. On the second postpartum day a bullous lesion developed on the plantar aspect of the left foot (Fig. 1), followed 4 weeks post partum by flare-up of the disease with vesiculobullous lesions (Fig. 2). She responded to 30 mg of oral prednisone that was gradually tapered. The diagnosis



Fig. 1. Dermatologic lesions of foot and leg in herpes gestationis.



Fig. 2. Dermatologic lesions of hand and arm in herpes gestationis.

of herpes gestationis was confirmed by skin biopsy, which revealed a subepidermal blister with perivascular infiltrate of eosinophils. The patient was a known case of recurrent herpes gestationis in which the diagnosis was confirmed by skin biopsy in the first pregnancy. Her history was significant for vesiculobullous lesions in all her previous viable pregnancies. In each pregnancy the onset of the disease was later and the severity was lessened. She required 120 mg/day oral prednisone in the first two viable pregnancies and 30 mg/day in subsequent pregnancies. The first pregnancy ended in a term stillbirth, allegedly the result of a cord accident.

Studies of the immune system of this patient in her first pregnancy were reported by Bonagura et al.[1] in *Human Immunology*. High titers of anti-HLA antibodies were detected then. Histolopathologic examination of the placenta in the last three pregnancies, while the patient was under our care, revealed varying degrees of villositis, with villi infiltrated with lymphocytes, plasma cells, and histiocytes.

Comment

The incidence of herpes gestationis varies from 1 in 4000 to 1 in 50,000, with a lower incidence when confirmed by immunofluorescence studies.[2] Recurrence in subsequent pregnancies is common and largely expected; however, postpartum onset of the disease is less common (14.63%).[2] Recurrence of the disease and postpartum flare-up, as in this patient, may indicate persistence of circulating autoantibodies. Flare-up of the disease also has been reported with establishment of menstruation in the postpartum period and with the use of oral contraceptives.

Although a significant increase in fetal morbidity has been reported in patients with herpes gestationis,[3, 4] such an association is questioned by others.[2] Shornick[2] attributed this disparity to the fact that the study of Lawley et al.[3] was based on literature review; hence it was more likely to include patients with more complicated disease and consequently poor fetal outcome.

Shornick's cases (no cases of adverse fetal outcome) were collected from immunofluorescence laboratory results, therefore possibly with less likelihood of selection bias. In the series of Holmes and Black[4] there was a wide referral base and all cases were included, creating a fair representation of fetal morbidity. In the patient described here, the first pregnancy ended in a term stillbirth. In subsequent pregnancies she underwent close observation, including antepartum fetal monitoring, and she received prednisone. Furthermore, analysis of the time of onset of lesions, severity of disease, placental pathologic condition, and fetal outcome in this patient showed that smaller babies occurred in the pregnancies in which placentas were severely affected. Evidence of villositis with lymphohistiocytic infiltration in the placenta, despite the absence of characteristic skin lesions in the antepartum period, shows that placental lesions may precede skin lesions.

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Transvaginal color Doppler imaging in the assessment of uteroplacental blood flow in the normal first-trimester pregnancy

Richard Jaffe, MD, and Steven L. Warsof, MD

Chicago, Illinois

Characteristic changes of low resistance and high diastolic blood flow velocity were identified by Doppler studies starting in the early phase of the second trimester. These changes were attributed by some authors to trophoblastic invasion of the uterine vasculature converting the uterus into a low-resistance organ. Because of technical limitations previous studies were confined to the uterine artery and its main branches. With the development of color Doppler and transvaginal ultrasonography we can now identify blood flow in various small vessels in the placental bed. This study was performed to assess the ability of color Doppler ultrasonography to evaluate subtrophoblastic blood flow by color identification in the early phase of normal pregnancy from 5 to 9 weeks' gestation. Results show that the characteristic increase in diastolic blood flow is evident as early as 5 weeks' gestation. (Am J Obstet Gynecol 1991;164:781-5.)

Key words: Color Doppler ultrasonography, transvaginal ultrasonography, uteroplacental blood flow

The introduction of Doppler technology into obstetrics gave us the opportunity for noninvasive evaluation of uteroplacental and fetal circulation in both normal and abnormal pregnancies.[1-8] The association between abnormal Doppler studies and both maternal and fetal disease has been suggested by several authors.[9-14] Doppler studies of uterine and umbilical arteries in normal pregnancies showed a progressive increase in diastolic blood flow starting early in the second-trimester.[2, 5, 6, 9, 10, 15] This reflects a fall in peripheral resistance postulated to be caused by the trophoblastic invasion of the uterine vasculature converting the uterus into a low-impedance organ.[16] With the recent introduction of high-frequency vaginal probes with color Doppler imaging, blood flow within the smallest arteries around the trophoblast can be detected and studied. This prospective study was undertaken to assess blood flow within the smallest arteries around the trophoblast early in the first trimester of normal pregnancies to try to elucidate the timing and magnitude of the changes that cause the decrease in peripheral vascular resistance.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Illinois at Chicago, College of Medicine.
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Reprint requests: Richard Jaffe, MD, University of Illinois at Chicago, Department of Obstetrics and Gynecology, 840 S. Wood St., Chicago, IL 60612.
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Table I. S/D ratios and resistance indexes of the study population

Gestational cases		*S/D ratio (±SD)*	*Resistance index (±SD)*
Week	*No.*		
5	6	2.52 ± 0.40	0.59 ± 0.00
6	8	3.23 ± 0.78	0.67 ± 0.08
7	10	3.28 ± 0.62	0.68 ± 0.05
8	10	2.90 ± 0.77	0.63 ± 0.11
9	10	2.42 ± 0.36	0.56 ± 0.05

S/D, Peak systolic to end-diastolic ratio.

Material and methods

Fifty healthy women were included in the study. All had at least one previous normal delivery. Their menstrual cycles were regular and all had accurately dated gestations by ultrasonography and dates. Included were only pregnancies in which heart activity could be detected and only those that progressed normally into the second trimester. The scans were performed with an Aloka SSD 680 scanner (Aloka Ltd., Japan), with the use of a 5 mHZ vaginal transducer with color and pulsed-wave Doppler. Ten women of each gestational age, 5 to 9 weeks, were studied only once. Flow around the trophoblast was identified by the appearance of color and two to four Doppler measurements from different sites were performed at each examination. The peak systolic to end-diastolic ratio (S/D) and resistance index were calculated with the formula:

$$RI = (S - D)/S$$

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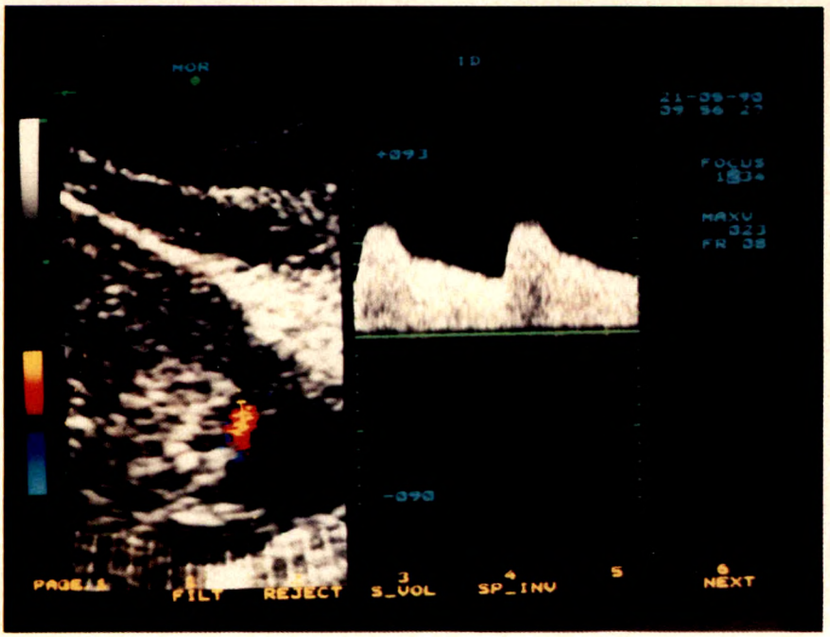


Fig. 1. Color Doppler imaging of 5-week gestation showing characteristic waveform of low-impedance organ.

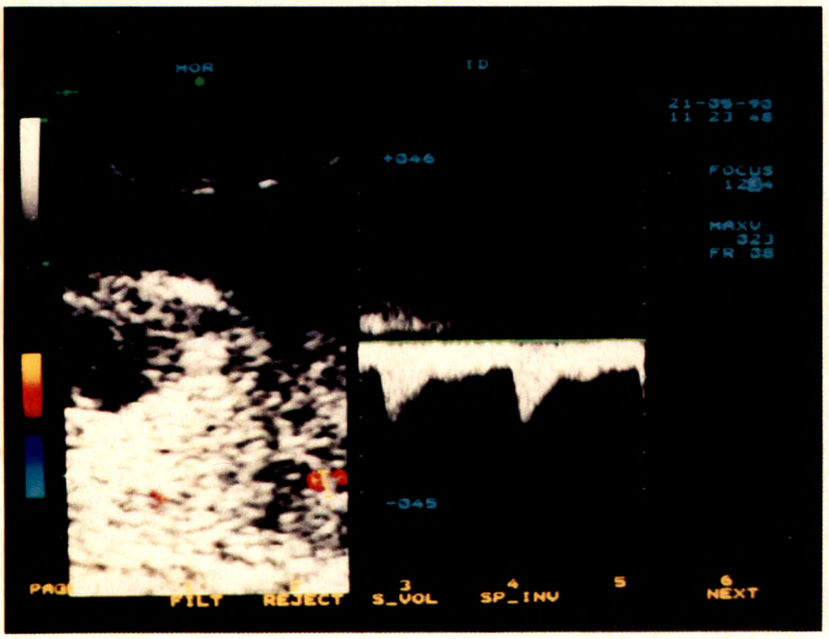


Fig. 2. Color Doppler imaging of 7-week gestation showing characteristic waveform of low-impedance organ.

where RI is resistance index, S is peak systolic and D is end-diastolic measurement. The difference between any two measurements on each examination did not exceed 8% and the mean was considered a good estimation of the actual ratios. On each measurement three waves were calculated and the mean S/D ratio and resistance index were noted. The final result was obtained by calculation of the mean S/D ratio and resistance index for all measurements at their respective gestational age.

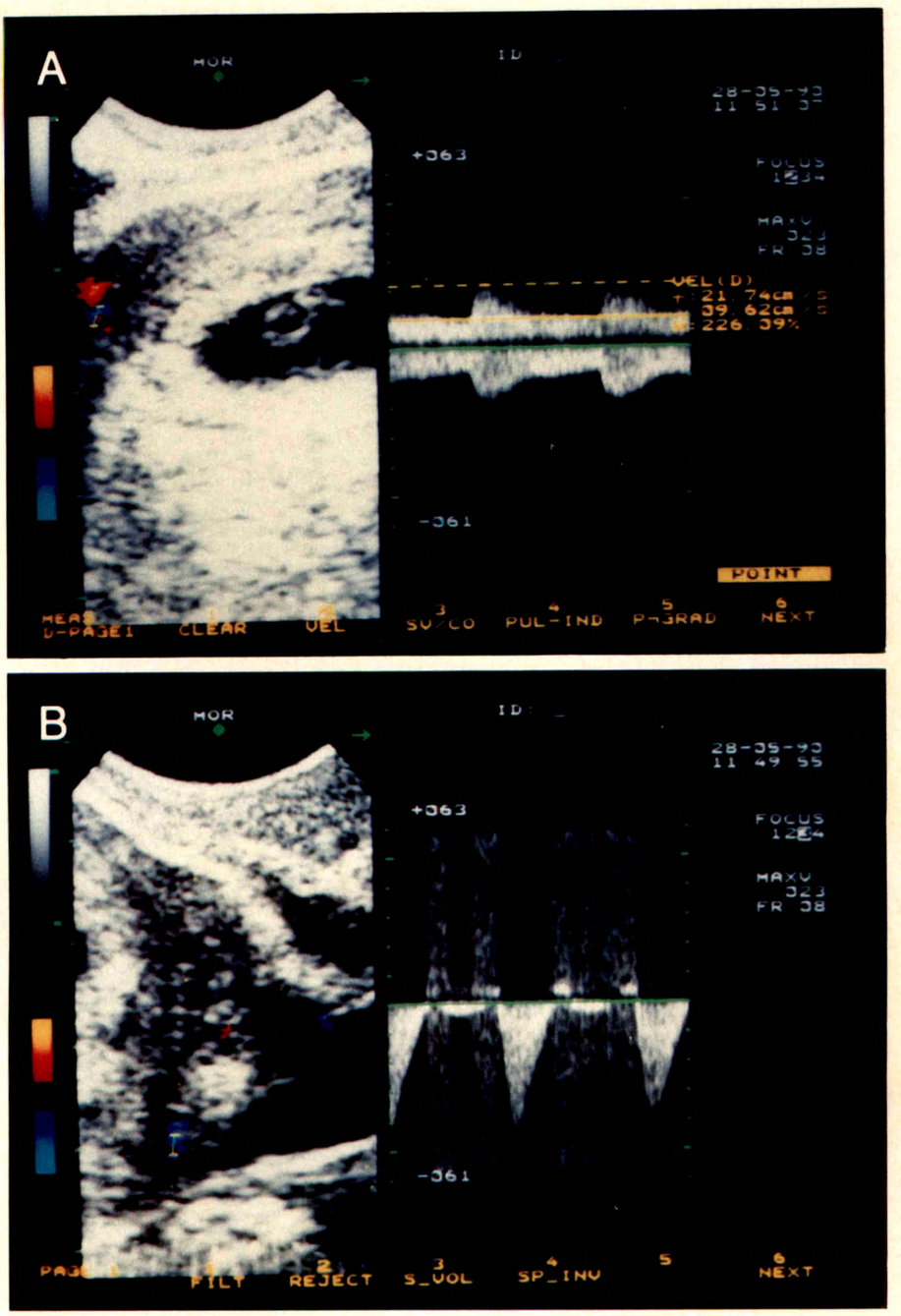


Fig. 3. Color Doppler imaging of trophoblastic flow **(A)** in 7-week pregnancy as compared with flow in uterine artery **(B)**.

Results

The changes in S/D ratios and resistance index with gestational age are depicted in Table I. The blood flow around the trophoblast could not be detected in four women at 5 weeks' gestation and in two women at 6 weeks' gestation. The flow was detected in all women from week 7 onward. Figs. 1 and 2 are examples of the blood flow detected by color imaging. Both figures show the high diastolic flow characteristic of a low-impedance organ. The results in Table I, with lower-than-anticipated S/D ratios and resistance index, show that the blood flow near the trophoblast has a prominent diastolic component as early as 5 weeks' gestation.

Comment

Doppler waveforms of the uterine artery in the normal nonpregnant uterus were previously described.[17] It was also shown by several authors that at some time in the second trimester the blood flow in the uterine arteries changes with the appearance of a high-diastolic component.[5, 6, 7, 15] One of the explanations behind this phenomenon is that at some time in the early pregnancy there is a trophoblastic invasion of the myometrial segments of the spiral arteries, causing the conversion of the uterus into a low-resistance organ with a high-diastolic blood flow.[20] Doppler technology provides the tool to follow the patterns and changes in the uterine circulation during pregnancy and any deviation from the normal can be observed. Both the continuous-wave Doppler system and the pulsed-wave system were used in previous studies of the uterine arteries.[1, 8, 15, 18] With both systems the ultrasonographic beam is directed toward the anatomic location of the uterine artery until the typical waveform pattern is obtained.[1, 15, 18]

Several studies reported the progressive decline in peripheral vascular resistance of the uterine vessels during pregnancy as shown by Doppler waveforms. These studies generally measured Doppler waveforms starting in the second trimester and reported the results obtained from the uterine arteries or their main branches. With the continuous-wave Doppler technique the signal can be from the sum of more than one artery,[18] and because it is a blind technique it is unlikely to measure the same location on consecutive examinations.[10, 15, 19] Differences of measurements as a result of placental location cannot be properly assessed by this method. With the pulsed-wave technique the blood flow must be measured from a vessel large enough to be seen by real-time ultrasonography to make it reproducible.

With the development of the color Doppler imaging this concept has changed. We can now identify blood flow wherever color is displayed by the Doppler signals and thus measure blood flow in vessels too small to be visualized.[20] Results of previous Doppler studies of the uterine arteries showed a high S/D ratio in early pregnancy[15] and a decrease toward the middle of the second trimester. This study for the first time of which we are aware shows a discrepancy between uterine blood flow measurements noted in previous studies and subtrophoblastic blood flow. We showed that the characteristic changes of the waveform occur at a much earlier stage of gestation at the level of the smallest arteries surrounding the trophoblast, as compared with the main uterine artery branches where similar changes do not occur until the second trimester. Fig. 3 shows an example of the Doppler measurement of the trophoblastic flow and the uterine artery flow illustrating this difference.

With the introduction of transvaginal color Doppler imaging it is hoped that it will be possible to detect diseases of placental structure and function in the first trimester. The role of this technique for determination of fetal viability and fetal or maternal disorders needs to be explored.

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Small-for-gestational-age birth: Maternal predictors and comparison with risk factors of spontaneous preterm delivery in the same cohort

Barbara Abrams, DrPH, RD, and Vicky Newman, MS, RD
Berkeley, San Francisco, and San Diego, California

Low birth weight, the primary predictor of infant mortality and morbidity, can be a result of shortened gestation (preterm delivery) or fetal growth retardation (small for gestational age). We examined the relationship between maternal characteristics and the risk of delivering a small-for-gestational age infant in 2228 women who participated in the University of California, San Diego Prenatal Nutrition Project between 1978 and 1988. A multivariate analysis indicated that significant risk factors for small for gestational age were cigarette smoking (odds ratio, 3.18), a low rate of maternal weight gain (odds ratio, 2.96), black ethnicity (odds ratio, 2.60), pregravid underweight (odds ratio, 2.36), Asian ethnicity (odds ratio, 1.88), primiparity (odds ratio, 1.85), and low maternal height (odds ratio, 1.63). These findings are contrasted with those previously published on preterm deliveries in the same cohort. We conclude that with the exception of black ethnicity and low maternal weight gain, different maternal characteristics were significantly associated with small-for-gestational-age and preterm birth in this population. (Am J Obstet Gynecol 1991;164:785-90.)

Key words: Risk factors, small for gestational age, low birth weight, weight gain

Low birth weight, the primary predictor of infant mortality and morbidity,[1-3] can be a result of shortened gestation (preterm delivery) or fetal growth retardation (small for gestational age, SGA). Because different factors may be associated with preterm delivery and SGA,[4] an understanding of the specific determinants of each is essential to design interventions to reduce their occurrence.

We described the relationship between maternal characteristics and spontaneous preterm delivery in a cohort of more than 2000 women who participated in the Prenatal Nutrition Project at the University of California, San Diego over an 8-year period.[5] Multivariate analysis of the data suggested that incompetent cervix (odds ratio, 6.15), use of illicit drugs during pregnancy (odds ratio, 3.90), exposure to trauma, surgery, or anesthesia during pregnancy (odds ratio, 3.47), maternal age <16 years (odds ratio, 2.91), black ethnicity (odds ratio, 2.10), low rate of maternal weight gain (odds ratio, 1.59) and short interpregnancy interval (odds ratio, 1.77) were significant risk factors of spontaneous preterm birth.

The objective of this article is to describe how ma-

From the Department of Social and Administrative Health Sciences, University of California, Berkeley; Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco; Wellstart/San Diego Lactation Program; and the Departments of Reproductive Medicine and Community and Family Medicine, University of California, San Diego.
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Reprint requests: Barbara Abrams, DrPH, RD, Public Health Nutrition Program, Department of Social and Administrative Health Sciences, School of Public Health, 419 Warren Hall, University of California, Berkeley, CA 94720.
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ternal characteristics related to the risk of SGA birth and to contrast these findings with those of our earlier study of preterm births in the same population.

Material and methods

The University of California, San Diego Prenatal Nutrition Project provided nutritional assessment and counseling to pregnant women considered at high risk of the development of nutritional problems during pregnancy. Information on demographics, diet, behavioral factors (such as cigarette smoking and use of illicit drugs), and medical and obstetric history were obtained by interview. Data on pregnancy course and outcome were obtained by chart review shortly after delivery. These data were entered into a computerized data base. Additional information on the cohort and on data collection procedures were previously published.[5]

For this analysis, the study group included all Prenatal Nutrition Project participants who were delivered of infants between January 1978 and December 1988 and whose pregnancies were uncomplicated by antepartum death, twin gestation, or major congenital anomalies.

Maternal characteristics selected for study were determined on the basis of previous reports of factors associated with SGA.[2, 3, 6-8] Prepregnancy weight was based on maternal recall at the first visit. Several studies suggest that self-reported weight reflects measured weight in women.[9-11] The single report on the accuracy of recalled pregravid weight, conducted in adolescents, concluded that although there was some variability in individual women, for the group as a whole, recalled prepregnancy weights correlated very closely with measured pregravid weight.[12] Maternal pregravid weight for height was based on the 1959 Metropolitan Insurance standards of desirable weight for height,[13] and divided into the following categories: underweight (<90%), ideal weight (90% to 119%), overweight (120% to 135%), and obese (>135%).

Total pregnancy weight gain was estimated by subtracting the prepregnancy weight from the last measured weight before delivery. Maternal weight gain was treated as a weekly rate of gain, estimated by dividing total weight gain by the gestational age at which the last weight measurement was made, minus 2 weeks subtracted from the denominator because gestational age was based on the last menstrual period. Low rate of gain was defined as <0.27 kg/week, which represents a total maternal weight gain of <10 kg at 40 weeks' gestation.

Each infant was classified as SGA (less than the 10th percentile), average for gestational age (AGA) (10th to 90th percentile), or large for gestational age (LGA) (greater than the 90th percentile) with the use of reference standards for birth weight for gestational age and sex published for California.[14] Gestational age was estimated primarily on the basis of maternal recall of the last menstrual period (LMP) and to a lesser extent on ultrasonographic or Dubowitz examinations. To ensure that infants were properly classified as preterm or term, the medical records of all infants with a birth weight <2750 gm or gestational age <38 weeks were reviewed by an obstetrician who specialized in maternal-fetal medicine.[5] However, no review was conducted for pregnancies that lasted >38 weeks' gestation or for infants who weighed >2750 gm. Because there is evidence that pregnancies are often erroneously classified as post dates on the basis of maternal LMP,[15, 16] infants with gestational ages >40 weeks were classified as SGA on the basis of the birth weight cutoff for 40 weeks (2944 gm for male infants and 2817 gm for female infants). Thus we used a conservative definition of SGA, and 19 infants who would be classified as post date SGA with the use of a gestational age of >40 weeks were classified as AGA. After classifying the infants according to birth size for gestational age, all LGA infants were eliminated from the analysis because LGA is associated with increased morbidity and mortality and has been shown to have different (and somewhat opposite) risk factors than those of SGA.[17, 18]

Differences in the distribution of maternal characteristics with the SGA and AGA groups were examined by cross tabulation with the use of χ^2 analysis. We then used a series of multiple logistic regression models to estimate the significance of each factor and its magnitude of risk in the prediction of SGA in this study group while controlling for the influence of the other factors. The analysis began with all factors in Table I included. Variables were then removed, one at a time, on the basis of both knowledge about the relationships between the factors and statistical significance, until only those variables remained that were significant predictors of SGA. Odds ratios, adjusted for the other significant predictors of SGA, and their 95% confidence intervals were calculated from the logistic coefficients and SEs estimated in the final model. Each odds ratio approximates the risk of SGA for women who were exposed to each risk factor, compared with the risk of SGA for women who were not exposed and adjusting for the other factors significantly associated with SGA birth in this study group. An odds ratio is statistically significant if its confidence interval excludes 1.0. Results for all analyses were considered significant if the two-tailed p value was <0.05.

Results

The ethnic distribution of mothers in the study group was 41% white, 32% Hispanic, 15% black, 11% Asian (primarily of Southeast Asian origin) and <1% other ethnicity. A total of 51% of the mothers were pri-

Table I. Incidence of SGA by sociodemographic, behavioral, and medical risk factors and comparison of the distribution of risk factors in the SGA (n = 219) and AGA (n = 2009) groups

	Incidence of SGA (%)*	*Distribution of risk factor*				
		SGA		*AGA*		
		n	*%*	*n*	*%*	*p Value*
Sociodemographic, behavioral factors						
Cigarettes (>10/day)	20.1	36	16.4	143	7.1	0.0001
Black ethnicity	17.0	58	26.6	283	14.1	0.0001
Hispanic ethnicity	6.5	46	21.1	660	32.9	0.0001
Not married	12.6	105	48.0	730	36.3	0.001
Illicit drug abuse	22.6	12	5.5	41	2.0	0.002
Low income	11.0	174	79.5	1410	70.2	0.004
Early prenatal care (first trimester)	7.7	63	28.8	759	37.8	0.009
Asian ethnicity	14.4	37	15.8	200	9.9	0.01
Unplanned pregnancy	10.7	139	63.5	1161	57.8	0.11
Short interpregnancy interval (<2 yrs of current birth)	11.0	29	13.2	235	11.7	0.50
Age						
≤15 yr	12.1	8	3.7	58	2.9	0.53
≥35 yr	8.2	9	4.1	101	5.0	0.55
Medical, obstetric, nutritional factors						
Low maternal weight gain (<0.27 kg/wk)	16.4	96	44.2	490	21.6	0.0001
Maternal underweight (<90% ideal)	16.5	64	27.2	325	16.1	0.0001
Low maternal height (<152 cm)	15.1	43	15.1	241	11.6	0.006
Hypertension†	14.9	33	15.1	188	9.4	0.007
Maternal overweight (120%-135% ideal)	5.8	15	6.4	246	12.2	0.008
Primiparity	8.3	125	57.1	973	48.4	0.01
Maternal obesity (>135% ideal)	8.4	19	8.1	207	10.3	0.29
Low maternal energy intake (<1200 kcal)	9.6	21	10.7	198	8.9	0.59
Anemia	9.9	139	59.2	1264	62.8	0.28
Spontaneous preterm delivery	9.1	12	5.5	120	6.0	0.60
Diabetes†	2.9	2	0.9	68	3.4	0.05

*Overall incidence of SGA birth in the sample was 9.8%.
†Includes both preexisting and pregnancy-induced.

miparous and 71% were low income. Mean maternal age was 24.0 years (SD, 5.5). Of the 2228 women in the study group, 219 were delivered of SGA infants and 2009 were delivered of AGA infants. Thus the incidence of SGA was 9.8%. Mean birth weight was 2510 gm (SD, 324.5) in the SGA group and 3350 gm (SD, 335.1) in the AGA group ($p < 0.0001$).

Table I shows that the SGA group had a higher frequency of women who were primiparous, black or Asian, low income, and unmarried. A higher percent of SGA mothers reported that they smoked cigarettes (>10 per day) and used illicit drugs. Compared with AGA mothers, SGA mothers were more likely to have short stature, begin pregnancy underweight for height, and have a low weekly rate of weight gain during pregnancy. There was significantly more hypertension (essential or pregnancy-induced) among SGA mothers. Alternatively, Hispanic ethnicity, prenatal care during the first trimester, maternal overweight, and diabetes were factors that occurred significantly less frequently among SGA mothers. Other risk factors including extremes of maternal age, unplanned pregnancy, short interpregnancy interval, maternal energy intake, and maternal obesity were not significantly related to SGA by univariate analysis.

Significant predictors of SGA from the multiple logistic model are shown in Table II. Two maternal characteristics were each associated with a tripled risk of SGA: smoking more than 10 cigarettes per day (odds ratio, 3.18) and a low weekly maternal weight gain during pregnancy (odds ratio, 2.96). The risk of SGA was more than doubled in women who were black (odds

Table II. Significant predictors of SGA by logistic regression analysis

Parameter	*Adjusted odds ratio*	*95% Confidence interval*
Cigarette smoking (>10/day)	3.18	2.06, 4.91
Low rate of maternal weight gain	2.96	2.17, 4.04
Black ethnicity	2.60	1.82, 3.71
Maternal hypertensive disorder	2.07	1.35, 3.18
Maternal pregravid underweight	2.36	1.67, 3.33
Asian ethnicity	1.88	1.19, 2.99
Primiparity	1.85	1.35, 2.64
Low maternal height	1.63	1.03, 2.44

ratio, 2.60), who began pregnancy with a low maternal weight for height (odds ratio, 2.36), or who had hypertension during pregnancy (odds ratio, 2.07). Other factors significantly related to SGA in the model were Asian ethnicity (odds ratio, 1.88), primiparity (odds ratio, 1.85) and low maternal height (odds ratio, 1.63).

Comment

Before discussing the results, several limitations in study design and execution should be noted. First, because it is a convenience sample, the Prenatal Nutrition Project Cohort cannot be directly generalized to other populations. Second, measurement of some of the variables including illicit drug use was not the original focus of the study. For example, rather than screening all women for illicit drug abuse in a standard fashion, drug abuse was diagnosed either when the pregnant woman admitted using drugs during the prenatal interview or when there was obvious behavioral or physical evidence of drug abuse during the course of pregnancy or at delivery. For this reason, we are less confident of results relating to these variables than others, such as maternal body size or ethnicity. Third, correct classification of infants as SGA is made on the basis of accurate estimation of gestational age, which is difficult in any study that is based on clinical data. We attempted to remedy this problem by reexamining the gestational ages of all small infants or those with gestational ages <38 weeks, and we used a conservative estimate of SGA for all infants who were delivered after 40 weeks' gestation.

The fourth potential limitation also relates to our ability to properly classify infants as SGA. The definition we used was based on a standard that identified sex-adjusted, gestational-age-specific cutoffs (below the 10th percentile) for fetal birth weight that were associated with the lowest perinatal mortality in California. Sex-adjusted standards were selected because although females have lower average birth weights than those of males, the difference is not associated with differences in the distribution of risk factors or in outcome.[4]

The question of whether to adjust for other maternal factors that are associated with birth weight, such as race, parity, or body size, is controversial.[19] For example, evidence that at the same low birth weight, black infants have a lower mortality rate than that of white infants, suggests that the fetal growth curve and its relationship to mortality may differ between white and black infants.[20] The fact that black infants are larger than white infants at early gestational ages and smaller at later ones[20, 21] suggests a biologic basis for differences in growth, and this has led to the suggestion that lower cutoffs for low birth weight and SGA be used for black women. However, there are also data that suggest that fetal growth is influenced more by maternal behavior than by genetics. For example, a study contrasted maternal health behaviors and birth weight in a population of black prenatal patients who were foreign-born and U.S.-born. The foreign-born mothers reported fewer behavioral risk factors for low birth weight; even after controlling for a variety of social, physical, and behavioral risk factors, their infants were on average 135 gm heavier than those of mothers born in the United States.[22] Thus the influence of race on birth weight is complex and still poorly understood. The use of race-adjusted standards can obscure the fact that blacks have disproportionately more low birth weight infants than do whites. In addition, standards that simultaneously adjust for race and sex or other maternal characteristics have not been published for California, thus the standards used in this study were simply sex-adjusted.

However, because the only sex-adjusted California standards were determined on the basis of the white, non-Spanish percentiles, if black (and possibly Southeast Asian) infants truly do better at lower birth weights than white infants, our analysis will have classified some infants in those racial groups as SGA when in fact they might be of appropriate size. If this is the case, the relationships we found between maternal risk factors and SGA are conservative, suggesting that if lower birth weight cutoffs were used, the associations would be even stronger.

In addition, because the standards used were based on population-based statistical data rather than clinical measurements, the definition of SGA cannot discriminate between infants of any race who were growth retarded or simply small, but well-grown.

Finally, because our study was observational in nature, we are unable to definitively say whether any risk factor was simply associated with or caused SGA birth.

Whereas these are important limitations, all the maternal characteristics that significantly predicted SGA in this cohort were previously linked with an increased

risk of SGA in other populations.[2, 3, 6-8] For this reason, we believe that the results reported here are relevant to other populations.

The first objective of this study was to evaluate the relationship between maternal characteristics and the risk of SGA in this cohort. First we examined the relationship between SGA and each factor one at a time. Then we entered all the factors simultaneously into the multivariate model to determine which of the maternal characteristics were independently related to SGA after controlling for the influence of the others. Cigarette smoking, black and Asian ethnicity, maternal hypertension, primiparity, low maternal height, pregravid weight, and weight gain each significantly predicted SGA in the multivariate model. These findings are similar to those recently reported in a Finnish case-control study of severely SGA neonates (defined as birth weight below the 2.5th percentile).[23] In that study of presumably white women only, the most important risk factors associated with SGA delivery were low maternal prepregnancy weight, history of previously being delivered of an SGA infant, low pregnancy weight gain, toxemia, and cigarette smoking.

The second objective of this study was to contrast the risk factors of SGA with those for preterm delivery. The analysis of preterm delivery for this population were reported in detail elsewhere.[5] Table III shows that although 13 different risk factors were significantly associated with either SGA or spontaneous preterm birth, only black ethnicity and a low weekly rate of maternal weight gain were associated with both.

We could identify only a few other studies that examined the relationship between maternal factors and both impaired fetal growth and preterm delivery in the same cohort. Villar et al.[7] studied a small group of women in rural Guatemala and reported that after multivariate analysis only a low maternal head circumference was significantly associated with intrauterine growth retardation, whereas maternal energy supplementation, protein supplementation, and low arm circumference were associated with preterm delivery.

Another study[24] examined how a variety of maternal risk factors predicted SGA and preterm delivery in a Canadian cohort. SGA was defined as birth weight below the 10th percentile but not preterm, whereas preterm was defined as birth before 37 weeks' gestation but not SGA. Although a variety of different maternal characteristics were associated with each outcome in the univariate analyses, after multivariate analysis, the only significant predictors of SGA were maternal height and prepregnancy weight, and the only significant predictors of preterm birth were maternal education and household income level.

Another study[25] contrasted 12 maternal risk factors of SGA and preterm delivery among 17,000 low-income women in Birmingham, Alabama, between 1983 and 1987. After multivariate analysis, SGA was significantly associated with black race, primiparity, increased maternal age, maternal smoking, low maternal height and weight, and a low rate of maternal weight gain during the last 20 weeks' gestation. Preterm delivery was significantly associated with black race, unmarried status, low and high maternal age, maternal smoking, low maternal weight, and both a low and high rate of maternal weight gain during the last 20 weeks' gestation. The authors suggested that the high rate of maternal weight gain associated with preterm delivery may have been a result of medical intervention in response to pregnancy-induced hypertension and its associated high weight gain, but they could not explain why maternal drug use was associated with a significantly decreased risk of preterm delivery.

In a study conducted in Brazil,[26] the authors found that both preterm delivery and intrauterine growth retardation were associated with low maternal weight, smoking, and few antenatal visits, whereas young maternal age was related to preterm birth and a low maternal education was related to growth retardation. Gestational weight gain was not reported. On the basis of these results, the authors concluded that low birth weight would be reduced with improvements in maternal nutrition, cessation of smoking, reduction in births to young women, and improved prenatal care.

These published reports support our finding that different maternal characteristics are associated with

Table III. Significant risk factors of SGA versus spontaneous preterm birth in singleton births uncomplicated by congenital anomalies

Factors	*SGA*	*Preterm delivery**
Sociodemographic		
Black ethnicity	X	X
Asian ethnicity	X	—
Age <16 yr	—	X
Behavioral		
Cigarette smoking (>10/day)	X	—
Illicit drug abuse	—	X
Nutritional		
Short stature (<152 cm)	X	—
Low pregravid weight for height (<90% ideal)	X	—
Low rate of maternal weight gain (<0.27 kg/week)	X	X
Medical, obstetric		
Primiparity	X	—
Short interpregnancy interval (<2 yr)	—	X
Incompetent cervix	—	X
Trauma, surgery, or anesthesia during pregnancy	—	X
Maternal hypertensive disorder	X	—

*Data presented in reference 5.

SGA versus preterm birth. The fact that the specific risk factors associated with either SGA or preterm delivery and their magnitude vary among the studies may be because of differences in methods (e.g., the way SGA or preterm births were defined, sample size, definition of the independent variables) or it may reflect real differences in risk among different populations.

In a critical meta-analysis of the published literature on determinants of low birth weight, Kramer[2, 4] concluded that in developed countries the most important maternal risk factors of intrauterine growth retardation included cigarette smoking, low maternal caloric intake or weight gain, low pregravid weight, primiparity, short stature, nonwhite race, and maternal history of low birth weight or previous delivery of a low-birth-weight infant. On the other hand, cigarette smoking, prior history of preterm birth or spontaneous abortion, low prepregnancy weight, and in utero exposure to diethylstilbestrol were the most important established maternal risk factors of preterm birth.

Low birth weight continues to be a significant public health problem. In this cohort, eight different common maternal risk factors of SGA were identified, confirming the results of other studies. In addition, our data suggest that different maternal factors are associated with SGA as compared with preterm delivery. Although only a few of the factors identified offer an opportunity for intervention, improvement of knowledge about the specific maternal characteristics that relate to SGA or preterm delivery may allow better identification or care of women who are at risk.

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Antisperm antibodies to sperm surface antigens in women with genital tract infection

Dean S. Cunningham, MD, PhD,[a] David L. Fulgham, BS,[b] David L. Rayl, MD,[a] Keith A. Hansen, MD[a] and Nancy J. Alexander, PhD[b]

Portsmouth and Norfolk, Virginia

Antisperm antibodies to sperm surface antigens in nulligravid women with primary upper genital tract infections were measured by the sperm mixed agglutination reaction assay. As many as 56% of women with a primary episode of pelvic inflammatory disease had antisperm antibodies. In addition, 69% of those women with no history of genital tract infection but with laparoscopic evidence of past pelvic infection had significant levels of circulating antisperm antibodies. Electroimmunoblots of sperm preparations probed with the sera of women who had either known or presumed upper genital tract infection revealed a uniformly recognized 69 kd antigen. In contrast, women with circulating antisperm antibodies before primary upper genital tract infection recognized up to five distinct sperm antigen determinants of 27, 54, 131, 146, and 174 kd. It is a distinct possibility that genital tract infections may lead to immunopotentiation of antisperm antibodies that could affect fertility. (AM J OBSTET GYNECOL 1991;164:791-6.)

Key words: Antisperm antibodies, genital tract infection, Western blot analysis, sperm surface antigens, infertility

Occasionally, both men and women have measureable antibodies that are directed against spermatozoan antigens. More commonly, couples with idiopathic infertility have antisperm antibodies.[1] Although the mechanism underlying antisperm antibody production is unknown, it has been proposed that it arises, among other ways, as a consequence of local inflammation.[2] Thus genital tract infections might serve as nonspecific immunopotentiators that lead to the production of antisperm antibodies. Therefore in this study we evaluated the relationship between genital tract infection and antisperm antibody production.

Material and methods

Patients. The study patients were nulligravid women 18 to 30 years old with no history of autoimmune disorders, gynecologic infections, or abdominopelvic surgery. They were seronegative for the human immunodeficiency virus. The patients were placed by diagnosis into one of five groups: (1) stage I pelvic inflammatory disease,[3,4] (2) stage II pelvic inflammatory disease,[3,4] (3) trichomonas vaginitis, (4) candida vaginitis, or (5) chlamydial cervicitis. The diagnoses of trichomonas and candida vaginitis were based on symptoms, character of vaginal discharge, vaginal pH, and the presence of trichomonads on saline solution preparation or hyphae and/or spores on potassium hydroxide preparation, respectively. Patients were considered to have chlamydial cervicitis when a cervical swab specimen was reactive in an enzyme-linked immunosorbent assay for *Chlamydia trachomatis* antigen, when >10 white blood cells per high-power field on a saline solution preparation of vaginal discharge was observed, and when pelvic examination results were insufficient to satisfy criteria for the diagnosis of pelvic inflammatory disease. Sexually active women who were free of genital tract infection and who met the previously described exclusion criteria were recruited as a control group during routine annual gynecologic examination. Two additional groups of patients consisted of patients with primary infertility who were undergoing diagnostic laparoscopy and were found to have either pelvic adhesive disease or hydrosalpinx without pelvic adhesive disease. Both written and verbal informed consent were obtained from all participants in an institution-approved protocol.

Five milliliters of venous blood was collected from each patient when she was admitted to the study and

From the Department of Obstetrics and Gynecology, United States Naval Hospital,[a] and The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School.[b]
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Reprint requests: Dean S. Cunningham, MD, Department of Obstetrics and Gynecology, United States Naval Hospital, Portsmouth, VA 23708.
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Table I. Antisperm antibody activity (immunoglobulin G) in women with known and presumed genital tract infections

Group	*Sperm mixed agglutination reaction test (No. and % positive)*	
	Serum	*Cervical mucus*
Stage I pelvic inflammatory disease (n = 28)		
Diagnosis	1 (3.5%)	0
Follow-up	13 (46.4%)	12 (42.9%)
Stage II pelvic inflammatory disease (n = 53)		
Diagnosis	3 (5.7%)	3 (5.7%)
Follow-up	24 (45.3%)	30 (56.6%)
Trichomonas vaginitis (n = 29)		
Diagnosis	4 (13.8%)	4 (13.8%)
Follow-up	5 (17.2%)	4 (13.8%)
Candida vaginitis (n = 25)		
Diagnosis	2 (8%)	1 (4%)
Follow-up	4 (16%)	3 (12%)
Chlamydia cervicitis (n = 32)		
Diagnosis	2 (6.3%)	3 (9.4%)
Follow-up	6 (18.8%)	7 (21.9%)
Pelvic adhesive disease (n = 29)	20 (69%)	15 (51.7%)
Distal tubal diseae (n = 16)	9 (56%)	3 (18.8%)
Control (n = 50)	4 (8%)	2 (4%)

again at the 4-week follow-up visit. Individual blood samples remained at room temperature for 1 hour for clot formation and for 2 hours at 4° C for clot retraction. The samples were centrifuged at 1200 *g* for 15 minutes. The resultant sera were inactivated at 56° C for 30 minutes and were stored at −80° C until they were used. One hundred microliters of cervical mucus was aspirated from each patient both at study entry and at follow-up evaluation. The cervical mucus was applied to a gel filtration column, and the eluate containing molecular weight fractions that correspond to immunoglobulins A and G was separately recovered, dialyzed overnight against a large volume of Dulbecco phosphate-buffered saline solution (DPBS) at 4° C, lyophilized, and reconstituted to the original volume in DPBS before it was stored at −80° C. No attempt was made to collect the follow-up samples of sera or cervical mucus at any specific time of the menstrual cycle, because the initial samples were obtained at different times of diagnosis.

Sperm mixed agglutination reaction test. The indirect sperm mixed agglutination reaction test was used to screen patient sera or cervical mucus for the presence of antisperm antibodies of the immunoglobulin G class, as described by Ackerman et al.[5] The serum or cervical mucus sample was considered positive when at least 40% of the motile sperm was bound to beads.

Sperm antigen extracts. Four semen samples were obtained from healthy donors who were shown to be negative for antisperm antibodies by the direct sperm mixed agglutination test.[5] Spermatozoa were collected by the swim-up technique[6] and resuspended at 60 × 10^6/ml in DPBS before being suspended in 50 mmol/L of Tris hydrochloride (pH 7.4) containing 10 mmol/L CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; Pierce Chemical Co, Rockford, Ill.). The sperm suspension was then placed in a Pyrex tube on ice and repeatedly sonicated in 10-second pulses at a setting of 20 kHz on a Biosonic III sonicator (Bronwill Scientific, Rochester, NY). Protease inhibitors (aprotinin 100 U/ml, 2 mmol/L pepstatin A, and 2 mmol/L phenylmethylsulfonyl fluoride; all from Sigma Chemical Co., St. Louis) were added to the extract, and the resulting suspension was rotated overnight at 4° C to allow for maximum solubilization. The sperm antigen preparation was then centrifuged at 85,000 *g* for 1 hour at 4° C, and the supernatant fluid containing solubilized sperm antigens was dialyzed overnight against a large volume of DPBS at 4° C. The protein concentration was determined by Bradford[7] analysis, and aliquots of the sperm extracts were stored at −80° C until they were used.

Electrophoresis and protein blotting. Sperm antigen extracts were analyzed by electrophoresis in polyacrylamide slab gels containing 0.1% sodium dodecyl sulfate with a 5% (wt/vol) polyacrylamide stacking gel and a 12.5% (wt/vol) polyacrylamide resolving gel in buffered Tris glycine, as described by Laemmli.[8] Samples containing sperm components were reduced and denatured by boiling in an equal volume of sample buffer (0.08 mol/L Tris hydrochloride [pH 6.8], 0.1 mol/L DL-dithiothreitol, 2.0% sodium dodecyl sulfate, and 10% glycerol) for 2 minutes before application to the gels.

The following standard proteins were used as molecular weight markers: myosin (200 kd), β-galactosidase (116.25 kd), phosphorylase b (92.5 kd), bovine serum albumin (66.2 kd), ovalbumin (45 kd), carbonic anhydrase (31 kd), soybean trypsin inhibitor (21.5 kd), and lysozyme (14.4 kd) (all from Bio-Rad Laboratories, Richmond, Va.).

Sperm antigen extracts resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis were transferred to nitrocellulose membrane strips. Nitrocellulose membranes (0.45 μm pore size; Bio-Rad Laboratories), sponges, and filter papers were preequilibrated in cold transfer buffer (25 mmol/L Tris hydrochloride [pH 8.3], 192 mmol/L glycine, and 20% [vol/vol] methanol) for 2 hours before use. Proteins were electrophoretically transferred from the gel onto 8-mm-wide strips of nitrocellulose at 30 V for 12 to 14 hours at 4° C. The efficiency of transfer was determined by staining selected strips that contained antigen and strips containing molecular weight markers in 0.1% (wt/vol)

amido black in 40% (vol/vol) methanol–2% (vol/vol) acetic acid. Strips were destained in 70% (vol/vol) methanol–7% (vol/vol) acetic acid. Under these conditions protein transfers were reproducible over a wide range of molecular weights.

Sperm antigen detection. Nitrocellulose strips containing sperm proteins resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis were analyzed by an indirect enzyme-labeled antibody assay. The strips were rinsed in DPBS after the electrophoretic transfer and incubated in DPBS containing 3% normal goat serum and 0.1% Tween 20 (all from Sigma Chemical Co.) for 2 hours as a blocking step. All incubations in the assay were performed in 25-well acrylic incubation trays (Bio-Rad Laboratories) that were placed in a high-humidity shaking water bath set at 37° C. The primary antisera used in the assay were the various patient sera samples (diluted 1:50 in DPBS containing 10% fetal bovine serum). Horseradish peroxidase–conjugated goat antihuman immunoglobulin A or G (Fisher Scientific Co., Springfield, N.J.), which was diluted 1:1000 in DPBS, was used as a second antibody. Strips were incubated in 10 ml of each of the antiserum preparations for a period of 1 hour with three 10-minute washes in DPBS containing 0.1% Tween 20 after each incubation. The strips were then developed in 120 ml of substrate solution (0.34 mmol/L 4-chloro-1-napthol dissolved in 20 ml of cold methanol and diluted in 100 mmol/L Tris–saline solution buffer that consisted of 0.04 mol/L Trizma hydrochloride, 9.7 mmol/L Trizma base, and 0.2 mol/L sodium chloride, pH 7.4; all from Sigma Chemical Co.) that was activated by the addition of 60 μl of 30% hydrogen peroxide.

Molecular weight determinations of the immunoprobed sperm antigens were made by polynomial regression analysis.

Results

The sperm mixed agglutination reaction test was used to screen women with primary genital tract infection for antisperm antibodies. The results of this test are presented in Table I. As many as 46% of women with stage I pelvic inflammatory disease had serum and cervical mucus antisperm antibodies of the immunoglobulin G class within 4 weeks of initial presentation and diagnosis. Similar findings were observed with women who had stage II pelvic inflammatory disease. A significant percentage of women with lower genital tract infections also had antisperm antibodies; however, antibody activity was demonstrated in <20% of all these patients tested. Women with no history of gynecologic infections but with laparoscopy verified pelvic adhesive disease or hydrosalpinx without adhesions had antisperm antibodies nearly 70% of the time. In our population the background antisperm antibody activity was

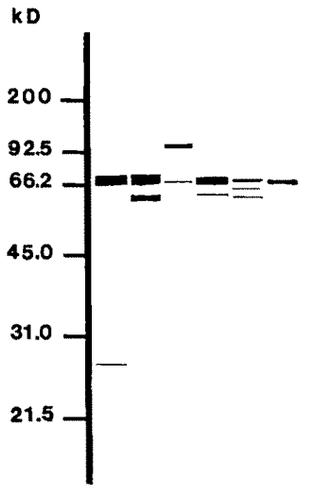


Fig. 1. Detection of sperm antigens by immunoblot analysis. *Lanes 1 to 3*, Sperm antigen detection by probe with sera from representative patients with stage I pelvic inflammatory disease. *Lanes 4-6*, Sperm antigen detection by probe with sera from representative patients with stage II pelvic inflammatory disease.

generally <10%, and no antisperm antibodies were demonstrated in the sera obtained from 17 women tested who had no history of exposure to spermatozoan antigens (data not shown). With the exception of the group of patients with distal tubal disease, the women studied had no differences in antisperm immunoglobulin G activity between the serum and cervical mucus. In this group approximately one third of patients with serum antisperm antibodies did not have measurable activity in the cervical mucus aspirate.

Fig. 1 is a display of immunoblots that are representative of patients with stage I (lanes 1 to 3) or stage II (lanes 4 to 6) pelvic inflammatory disease. A dominant 69 kd band was identified in all sera tested from these patients; no other constituent bands appeared uniformly. These results were found to be similar whether pooled semen or semen from the patient's current sexual partner only was used as a source of sperm antigens (data not shown). The sperm protein bands identified after the preparative gel was stained were similar, regardless of antigen preparation technique (freeze-thaw sonicate, biotinylated purification, NP-40 extraction, CHAPS extraction; data not shown).

As shown in Fig. 2, when sera from patients with antisperm antibody activity at the time of upper genital tract infection diagnosis were compared with paired sera at follow-up evaluation, there were numerous detectable bands, none of which included the 69 kd band identified in patients without preexisting antisperm antibody activity. The collective antigen bands from all patients migrated with molecular weight markers of 27,

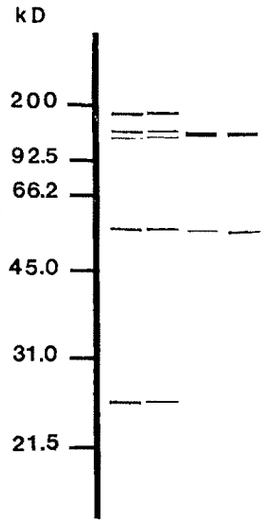


Fig. 2. Detection of sperm antigens by immunoblot analysis. *Lanes 1 and 2,* Acute and convalescent sera from representative patient with stage I PID and preexisting antisperm antibodies. *Lanes 3 and 4,* Acute and convalescent sera from representative patient with stage II pelvic inflammatory disease and preexisting antisperm antibodies.

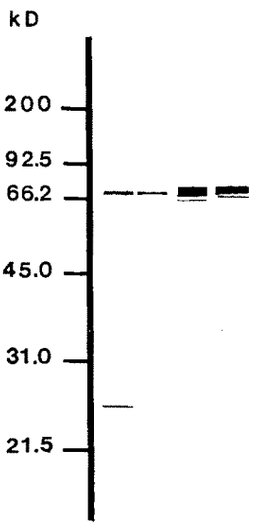


Fig. 3. Detection of sperm antigens by immunoblot analysis. *Lanes 1 and 2,* Sera from representative patients with pelvic adhesive disease. *Lanes 3 and 4,* Sera from representative patients with distal tubal disease.

54, 131, 146, and 174 kd. All five protein bands were detected in some (but not all) sera samples. In no case was the antigen band profile different in the serum obtained at diagnosis when compared with the serum obtained at follow-up evaluation.

Patients with presumptive evidence of a past upper genital tract infection at diagnostic laparoscopy had antigen band patterns that resembled those of patients with a primary upper genital tract infection (compare Fig. 3 with Fig. 1). The 69 kd antigen was consistently recognized; other antigens were not recognized consistently.

Fig. 4 is a collection of immunoblots from patients without a history of upper genital tract infection. Lane I shows the antigen band pattern of a woman with antisperm antibodies and no history of either upper or lower genital tract infection. When all patients were considered together, the antigen bands were restricted to the molecular weight ranges found in patients with preexisting antisperm antibody activity (Fig. 2). Lanes 2 and 3, 5 and 6, and 8 and 9 represent the antigen bands detected in the presentation and follow-up sera of patients with trichomonas vaginitis, candida vaginitis, and chlamydia cervicitis, respectively. These paired samples show antigens that are characteristic of women with preexisting antisperm antibody activity and no new antigen bands. Lanes 4, 7, and 10 represent the follow-up sera samples from patients with no preexisting antisperm antibody activity who were diagnosed with primary trichomonas vaginitis, candida vaginitis, and chlamydial cervicitis, respectively. Once again, no antigenic determinants stained other than the 27, 54, 131, 146, and 174 kd bands demonstrated in patients with preexisting antisperm antibodies (compare Fig. 4 with Fig. 2).

The lanes in Fig. 5 are comparisons of immunoglobulin isotypes in cervical mucus from patients without a history of genital tract infection (lanes 1 and 2), stage I pelvic inflammatory disease (lanes 3 and 4), and stage II pelvic inflammatory disease (lanes 5 and 6). All cervical mucus samples had been screened with the sperm mixed agglutination reaction test and were known to have antisperm antibodies of the immunoglobulin G class only before immunoprobe analysis for immunoglobulin A. The probed antigenic bands detected by antisera against immunoglobulins G and A differed in all samples tested and included many antigenic band molecular weights that were not detected in the sera samples (28, 58, 87, 96, 140, 201, 244, and 311 kd). No antigenic band was consistently detected, as was the case with the 69 kd band in women with upper genital tract infection or the five antigenic bands in women with preexisting antisperm antibodies or lower genital tract infections.

Comment

In this study we have demonstrated a relationship between genital tract infection and the production of antisperm antibodies in some (but not all) nulligravid

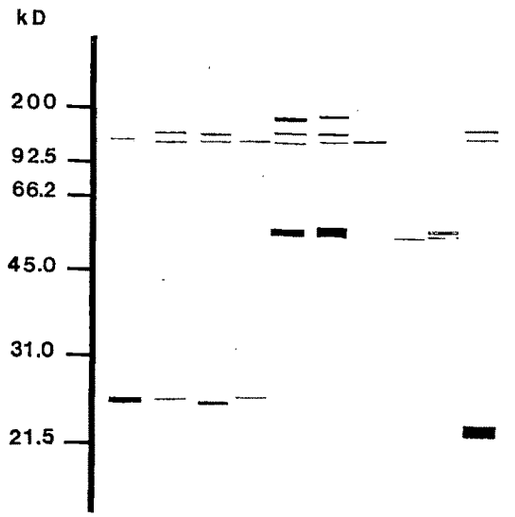


Fig. 4. Detection of sperm antigens by immunoblot analysis. *Lane 1,* Sera from representative patient with antisperm antibody activity and no history of genital tract infection. *Lanes 2 and 3, 5 and 6, and 8 and 9,* Acute and convalescent sera from representative patients with trichomonas vaginitis, candida vaginitis, and chlamydia cervicitis, respectively, as well as preexisting antisperm antibodies. *Lanes 4, 7, and 10,* Convalescence sera from representative patients with trichomonas vaginitis, candida vaginitis, and chlamydia cervicitis, respectively, with no preexisting antisperm antibodies.

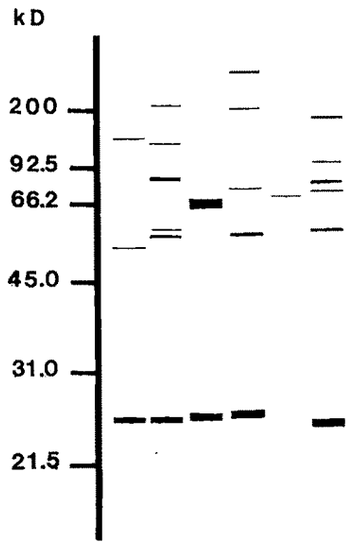


Fig. 5. Detection of sperm antigens by immunoblot analysis. *Lanes 1 and 2, 3 and 4, and 5 and 6,* Cervical mucus from representative patients with no history of genital tract infection, stage I pelvic inflammatory disease, and stage II pelvic inflammatory disease, respectively, in which an antiimmunoglobulin G *(lanes 1, 3, and 5)* or antiimmunoglobulin A *(lanes 2, 4, and 6)* secondary antibody was used.

women. This is not without precedent; Witkin and Toth[2] have shown that antisperm antibodies developed in a similar percentage of men with urethritis, and Blum et al.[9] described a very significant correlation between antisperm antibodies and antichlamydial antibodies in oral contraceptive users. We confirmed these general findings and also showed a striking difference in the characteristics of humoral responses generated against spermatozoa.

We have shown that in nearly 50% of women with a primary upper genital tract infection, antisperm antibodies develop against a restricted set of antigens. The immunodominant antigen is universally recognized as having a molecular weight of 69 kd. This pattern is distinct from that in women with lower genital tract infections who had a limited but overlapping repertoire of detected antigens. When they have been established, antisperm antibody specificities are apparently immutable, as evidenced by the group of women with preexisting antibody activity that antedated the primary genital tract infection. These findings suggest an underlying immunoregulatory event, and indeed Witkin[10, 11] has produced ample data to support a break in immunoregulation as the basis for antisperm antibody production in women. This concept may explain the failure to generate antisperm antibodies in some 30% of the female population (Cunningham DS, Fulgham DL, and Alexander NJ. Unpublished observations). It is difficult to determine how these findings translate into biologic significance because no bioassay exists that can relate antisperm antibody activity to a reproductive event, a determination of postinfection pregnancy rate in this group is as yet uncompleted, and the cause of antisperm antibody responses is not known nor is it understood why responses are not uniform. Even though the details have not been clarified, it is widely accepted that antisperm antibodies, whatever their cause(s), result in a diminished fertility capacity.[6, 12, 13]

We intend to test specific sperm antigens for their immunogenicity in vitro by a panel of lymphocytes from women who have no measurable antisperm antibody activity to facilitate the detection of a meaningful antigen to which to direct monoclonal antibody production. This line of investigation may help in the refinement of antisperm antibody detection methods. Selection of an appropriate screening assay for antisperm antibodies is difficult yet important for both clinicians and researchers. We chose the sperm mixed agglutination reaction test from the variety available[14] for its simplicity and apparent suitability.[1, 5] The accuracy of the test was substantiated when the presence of antisperm antibodies in each positive serum and cervical mucus sample was confirmed by immunoprobe analysis, or the absence of these antibodies was similarly

demonstrated, and when antisperm antibody activity was lost after simple absorption experiments using swim-up sperm or biotinylated sperm surface antigens. Neither a specific antigen(s) nor a superior antibody detection assay exists, although both are requisite to an understanding of the significance of antisperm antibody production and the potential manipulation of the female immune response to spermatozoa for the purpose of infertility reduction and potential immunocontraception.

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Genotyping of macerated stillborn fetuses

Catherine Derom, PhD,[a] Robert Vlietinck, MD,[a] Robert Derom, MD,[a] Charles Boklage, PhD,[c] Michel Thiery, MD,[b] and Herman Van den Berghe, MD[a]

Leuven and Ghent, Belgium, and Greenville, North Carolina

It is generally impossible to collect blood or to culture tissue from a macerated stillborn fetus. Accurate genotyping of such a fetus may, however, be critical for the diagnosis of genetic diseases and appropriate genetic counseling. In the East Flanders Prospective Twin Study, placental tissue of twin and triplet sets, in some of which one or both members were stillborn and macerated, has been stored at −20° C. Of all these fetuses, sex and zygosity could be determined accurately on the placental deoxyribonucleic acid. We tested the possibility of nongenetic changes in deoxyribonucleic acid that result from maceration or tissue degradation over time in storage on placental samples from monochorionic twins in which only one member was stillborn and macerated. The deoxyribonucleic acid variants in these monozygotic twins were identical whether or not either cotwin was macerated. Thus deoxyribonucleic acid variants can be determined accurately on the placental tissue of macerated fetuses, even after prolonged freezing. (Am J Obstet Gynecol 1991;164:797-800.)

Key words: Genetic counseling, genotype, stillbirth, twins, zygosity

Recombinant deoxyribonucleic acid (DNA) methods and the analysis of DNA polymorphisms have become increasingly important for carrier detection and prenatal diagnosis of a growing number of genetic diseases.[1] Prenatal diagnosis is possible in the embryo or the fetus because the DNA defect is present in every cell, including those of chorionic villi. Numerous DNA restriction fragment length polymorphisms have now been described. Many of these result from the variable presence of particular restriction enzyme recognition sites giving rise to two or more alleles at each locus.[2, 3] Polymorphism may also be generated by length variation in DNA fragments from hypervariable regions consisting of a variable number of tandem repeats. These regions are often highly informative for genetic analyses because of the multiallelic character of such loci.[4, 5] More recently midisatellite and minisatellite DNA probes have been described which hybridize to many variable numbers of tandem repeats simultaneously, either clustered at a single locus[6, 7] or scattered throughout the genome.[8, 9] Such probes show a highly polymorphic pattern in the population and give rise to restriction fragment length polymorphism patterns that may be unique to the individual (DNA "fingerprints"). All of these types of DNA polymorphisms have been shown to be inherited in mendelian fashion.

Table I. Human DNA probes

Probe	*Restriction enzyme*	*Description*	*Chromosome assignment*	*References*
L 1.22	*Bg* III	2 Alleles	1	3
L 2.30	*Bg* III	2 Alleles	2p25	3
pAW101	*Eco* RI	VNTR	14q32	4
pYNH24	*Taq* I	VNTR	2	5
pYNA23.1	*Taq* I	VNTR	?	5
hMF1	*Taq* I *Pst* I	Midisatellite	1pter	6, 7
pUC15	*Hin* fI *Hae* III	Minisatellite	Multiple	8, 9
pUC6.3	*Hin* fI *Hae* III	Minisatellite	Multiple	8, 9
P1	*Hae* III	Y specific at high stringency	Yq12	14
49f	*Eco* RI	Y specific at high stringency	Yq11.2	14, 15

VNTR, Variable number of tandem repeats.

DNA can still be extracted from tissues that have been stored for long periods of time.[10] Because we have stored placental tissues from a number of macerated stillborn fetuses, we are able to test the use of placental DNA polymorphisms for their genotyping. Cotwins known to be monozygotic by virtue of monochorionicity, but discordant for stillbirth and maceration, pro-

From the Centre for Human Genetics, Catholic University of Leuven,[a] the Department of Obstetrics, State University of Gent,[b] and the Genetics Program, East Carolina University School of Medicine.[c]
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Reprint requests: Catherine Derom, PhD, Centre of Human Genetics, Catholic University of Leuven, Herestraat 49, B-3000 Leuven, Belgium.
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Table II. Genotyping of macerated stillborn fetuses

Identification No.	*Status at birth*	*Fetal membranes*	*Birth weight (gm)*	*AP*	*L1.22,* L2.30*	*pAW101,* pYNH24, pYNA23, hMF1*	*pUC15,* pUC6.3*	*P1,* 49f*	*Zygosity after genotyping*
G875									
1	Ma.	DC	3000	†	‡	†	†	F	DZ
2	Ma.		2500					F	
G1005									
1	L	DC	1700	‡	–	‡	‡	F	pMz > 0.99
2	Ma.		1400					F	
G1126									
1	Ma.	MC	900	‡	–	‡	‡	M	MZ
2	L		900					M	
G1275									
1	L	MC	3000	‡	‡	‡	‡	M	MZ
2	Ma.		2600					M	
G1323									
1	Ma.	MC	±100	‡	‡	‡	‡	M	MZ
2	L		2650					M	
G1350									
1	N	MC	1500	‡	‡	‡	‡	F	MZ
2	Ma.		35					F§	
G1556									
1	L	DC	3350	‡	–	‡	‡	M	pMZ > 0.99
2	Ma.		2000					M	
G1789									
1	L	MC	2000	‡	‡	‡	‡	F	MZ
2	Ma.		1400					F	
G1932									
1	Ma.	DC	125	‡	‡	‡	‡	M	pMZ > 0.99
2	N		150					M	
G2021									
1	Ma.	?	80	–	‡	‡	‡	M	pMZ > 0.99
2	S		110					M	
G2247									
1	L	MC	3750	‡	‡	‡	‡	F	MZ
2	Ma.		250					F§	
G2293									
1	Ma.	MC	1800	‡	‡	‡	‡	F	MZ
2	Ma.		1800					F	
G2314									
1	L	DC	4030	‡	–	†	†	F	DZ
2	Ma.		2000					M	
G2445									
1	L	MC	3200	‡	‡	‡	‡	F	MZ
2	Ma.		2500					F	
G2542									
1	Ma.	MC	700	–	‡	‡	‡	M	MZ
2	Ma.		1050					M	
G2710									
1	L	DC	2370	‡	–	†	†	M	DZ
2	Ma.		850					M	
T51									
1	L	TC	1870	1,3‡	1,2†	1,2†	1,2†	M	TZ
2	Ma.		345	2,1†	2,3‡	2,3‡	2,3†	M	
3	Ma.		110	2,3†	1,3†	1,3†	1,3†	F§	
T58									
1	Ma.	TC	910	1,2‡	–	1,2‡	1,2‡	F	pMZ > 0.99
2	L		1090	2,3‡	–	2,3‡	2,3‡	F	
3	L		900		–			F	

G, Gemelli; *T*, triplet. Status at birth: live-born *(L)*, neonatal death *(N)*, fresh stillbirth *(S)*, macerated stillbirth *(Ma.)*. *F*, Female; *M*, male; *MC*, monochorionic; *DC*, dichorionic; *TC*, trichorionic; *MZ*, monozygotic; *DZ*, dizygotic; *TZ*, trizygotic. *pMZ*, Probability of monozygosity; *AP*, placental alkaline phosphatase isozymes.

*The DNA probes used are described in Table I.

†Different genetic markers.

‡Identical genetic markers.

§Sex unknown before DNA genotyping.

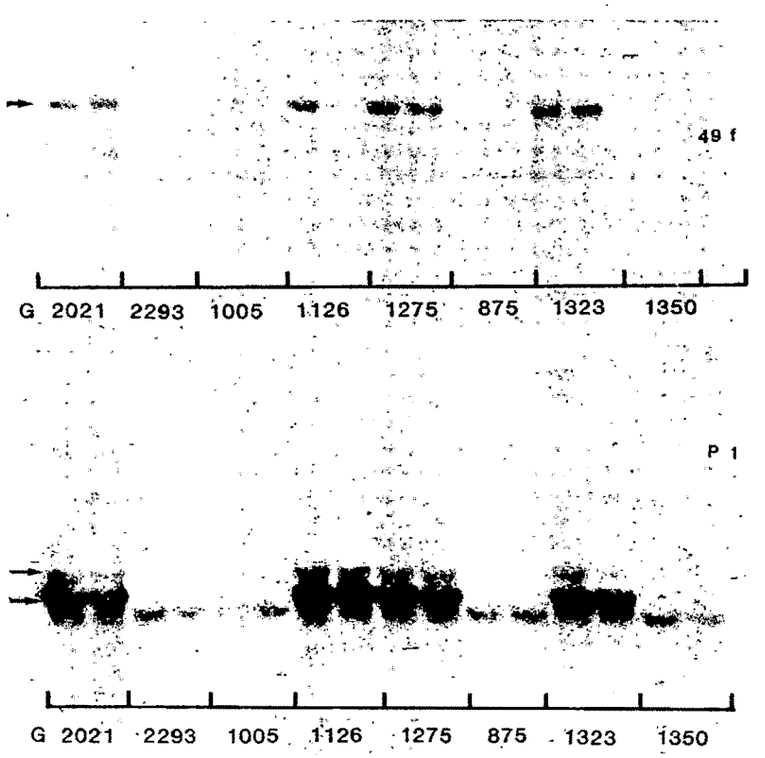


Fig. 1. Hybridization of Y-chromosome-specific probes to the genomic blots of macerated stillborn fetuses. The *Eco*RI blot and the *Hae* III blot were hybridized with the 49f unique sequence probe and the repetitive P1 probe, respectively.[14] Both filters were washed under stringent conditions (0.1 × sodium saline citrate at 68° C). The Y-specific bands are indicated.

vide a unique opportunity to test for any DNA changes resulting from maceration or to any degradation during frozen storage.

Material and methods

The East Flanders Prospective Twin Study was begun on July 1, 1964. To date more than 3300 multiple births are registered.[11] Zygosity diagnosis is based on fetal sex, structure of the fetal membranes, umbilical cord blood groups, placental alkaline phosphatase isozymes, and, more recently, DNA polymorphisms as well. Unlike-sexed twin pairs and same-sexed dichorionic twin pairs with at least one different genetic marker are classed as dizygotic. Monochorionic twin pairs are classed as monozygotic. For same-sexed dichorionic twin pairs with identical markers, we have calculated the probability of monozygosity on the basis of the allele frequency of all tested genetic markers.[12]

The 16 twin pairs and the two triplet sets used for DNA typing in this study were selected at random from the sets in which at least one member was macerated and stillborn. The DNA probes used are described in Table I. PUC15 and PUC6.3 were derived from probes 15.1.11.4 and 6.3, respectively, of Jeffreys et al.[8] Placental DNA extraction, restriction enzyme digestion, and Southern blot hybridization were as previously described.[13] The placental specimens had been stored in a regular freezer (−20° C) for a median time of 7.5 years (range, 8 to 163 months).

Results

Table II summarizes the results of the placental DNA analysis of the macerated stillborn fetuses with the DNA probes listed in Table I. In all of these fetuses the Y-specific probes[14, 15] allowed for the determination of sex (Fig. 1). The influence of tissue degradation on the restriction fragment length polymorphisms was evaluated on the placentas of nine monochorionic twin pairs. In seven of them only one member was a macerated stillborn fetus, so that possible influence of in utero tissue degradation on the restriction fragment length polymorphisms could be detected. The DNA variants and the alkaline phosphatase pattern of monochorionic cotwins were indistinguishable, whether the subjects were stillborn and macerated or living.

Zygosity could be diagnosed as well, i.e., when the

diagnosis relied on the analysis of the placental genetic markers only. The twin pairs G875, G2314, G2710, and the set of triplets T51 proved to be dizygotic and trizygotic, respectively, while the sets G1005, G1556, G1932, G2021, and T58 reached a probability of monozygosity of more than 0.99.

Comment

Even after prolonged freezing the analysis of DNA variants on the placental tissue of macerated stillborn fetuses can be performed accurately. In three cases where sex was unknown at birth because of extreme maceration (child 2 of G1350 and G2247 and child 3 of T51), we were able to determine sex by DNA typing.

Similarly, zygosity diagnosis was obtained in circumstances when, because of maceration, no blood could be sampled for blood group determination, either from the umbilical cord or from other sites.

Given that monozygotic twins have identical restriction fragment length polymorphisms,[9, 13] these analyses performed on monochorionic (therefore monozygotic) twin pairs, one of whom was live-born and the other stillborn and macerated, allow for the demonstration that the placental DNA pattern is identical in the living and macerated cotwins. Placental DNA does not deteriorate detectably after intrauterine death.

With the exception of any somatic mutations and the tissue-specific methylation patterns, the placental and fetal DNA have the same restriction fragment length polymorphisms.[16] Although some authors argue for DNA rearrangements in trophoblast[17] via mitotic recombination, we did not observe any variation in the placental DNA pattern (DNA fingerprints included) of our monozygotic twin pairs.

The use of placental DNA for the genotyping of a macerated stillborn fetus has two main advantages:

1. Placental tissue is very rich in DNA and can be stored at −20° C for a long period of time. If immediate genotyping is not possible or if any unusual DNA variant is found, enough DNA would be available to provide a new standard for this new variant.

2. It is now feasible to use random human chromosome-specific sequences to establish a linkage map for the entire human genome.[18] This should permit diagnosis for many genetic diseases by linkage analysis in the macerated fetus. As more and more loci for genetic diseases become mapped and known, genotyping macerated fetuses is important for diagnosing some of the causes of antenatal death and providing genetic counseling.

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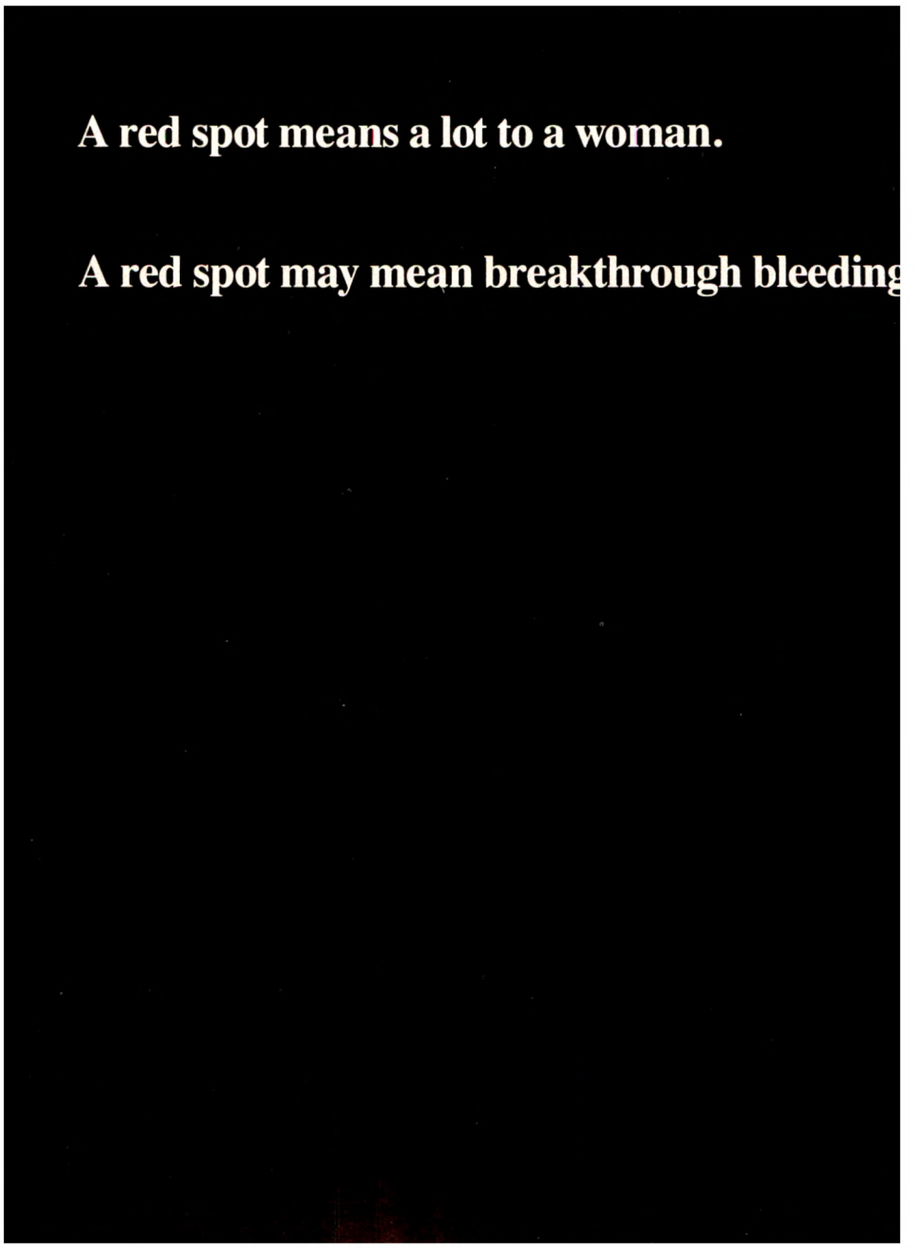
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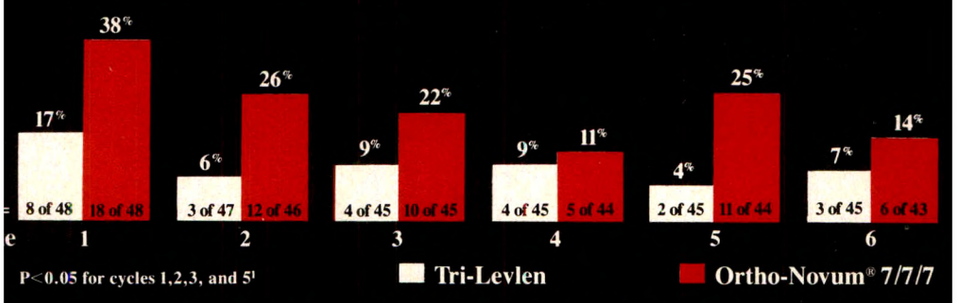
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MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.
RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.
ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.
PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.
2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.
3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.
4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.
5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.
6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.
7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.
8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.
9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.
10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.
11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.
12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

TRI·LEVLEN®
Levonorgestrel and ethinyl estradiol tablets–
Triphasic regimen

Information for the Patient—See Patient Package Labeling
Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.
Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.
Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."
Nursing Mothers—See "Warnings."
Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.
There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.
The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.
The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.
Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.
Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.
For full details on dosage and administration see prescribing information in package insert. 60611-1 5/86

Reference: 1. Data on file, Berlex Laboratories, Inc.

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Weight gain by gestational age in both black and white women delivered of normal-birth-weight and low-birth-weight infants

Diana B. Petitti, MD,[a] Mary S. Croughan-Minihane, PhD,[a] and Robert A. Hiatt, MD, PhD[b]
San Francisco and Oakland, California

Although adequate maternal weight gain during pregnancy is widely acknowledged as a desirable goal, a contemporary standard for weight gain over the entire period of gestation based on a low-risk U.S. population has not been available. Information about usual weight gain in early pregnancy was particularly lacking. In a study monitoring various aspects of prenatal care in women in a population-based case-control study of low birth weight, we collected information on weight gain at various gestational ages in the 281 women who had prenatal care and were delivered of infants at a prepaid health plan facility where standardized records were kept. In white women who were delivered of infants who weighed ≥3000 gm, average weight gain was 5.3 pounds at 9 to 10 weeks' gestation, 10.5 pounds at 11 to 20 weeks, 24.2 pounds at 21 to 30 weeks, and 35.1 pounds at 31 to 40 weeks. Weight gain among black women delivered of infants who weighed ≥3000 gm was 4.2 pounds at 9 to 10 weeks' gestation, 14.8 pounds at 11 to 20 weeks, 24.5 pounds at 21 to 30 weeks, and 33.9 pounds at 31 to 40 weeks. Weight gain was highly variable at all gestational ages in both black and white women. Weight gain in both black and white women delivered of infants with weights ≥3000 gm was linear at a rate of about 0.66 pounds per week from 8 through 20 weeks' gestation and linear at a rate of 1.06 pounds per week after week 20. (AM J OBSTET GYNECOL 1991;164:801-5.)

Key words: Low birth weight, weight gain

Maternal weight gain accounts for the largest proportion of variation in infant birth weight among term infants and the second largest proportion among infants born at all gestational ages.[1] In turn, infant birth weight is strongly associated with infant morbidity and mortality. Although there is general agreement that pregnant women should gain at least 15 pounds, relatively little is known about patterns of weight gain during early pregnancy, and there is no agreement on a standard of weight gain by gestational age. There are a large number of previous studies of maternal weight gain pattern in pregnancy,[1-16] but nearly all[2-14] were conducted during periods of prescribed weight restriction or were conducted in countries other than the United States. Additional limitations of most previous studies are restriction of the study population to poor women, lack of information on sample selection, and failure to consider infant birth weight.

In this study, routine prenatal care records were reviewed to obtain information on prepregnancy weight and weight gain for a group of both black and white middle-class women delivered of low-birth-weight (<2500 gm) and normal weight (≥3000 gm) infants. We used these data to address three questions. First, what is the pattern of weight gain in a group of women delivered of infants under conditions of unrestricted weight gain? Second, does the pattern of weight gain differ between low-risk black women and white women delivered of infants of normal weight? Third, does the pattern of weight gain differ between women delivered of low-birth-weight and normal-birth-weight infants?

From the Department of Family and Community Medicine, University of California at San Francisco, School of Medicine,[a] and the Division of Research, Kaiser Permanente Medical Care Program, Oakland.[b]
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Reprint requests: Diana B. Petitti, MD, AC-9, Box 0900, San Francisco, CA 94143.
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Methods

We did a population-based case-control study of low birth weight in Alameda County, California, which has been described previously.[16] Briefly, we used birth certificates to identify both black and white low-birth-weight (<2500 gm) singleton infants without anomaly and an equal number of both black and white singleton infants of normal birth weight (≥3000 gm) born in Alameda County to residents of Alameda County in 1987. There were 500 black patients, 500 black control patients, 268 white patients, and 268 white control patients.

For the current study, we reviewed the medical records of the 308 women among these cases and controls who were delivered of infants at Kaiser Permanente

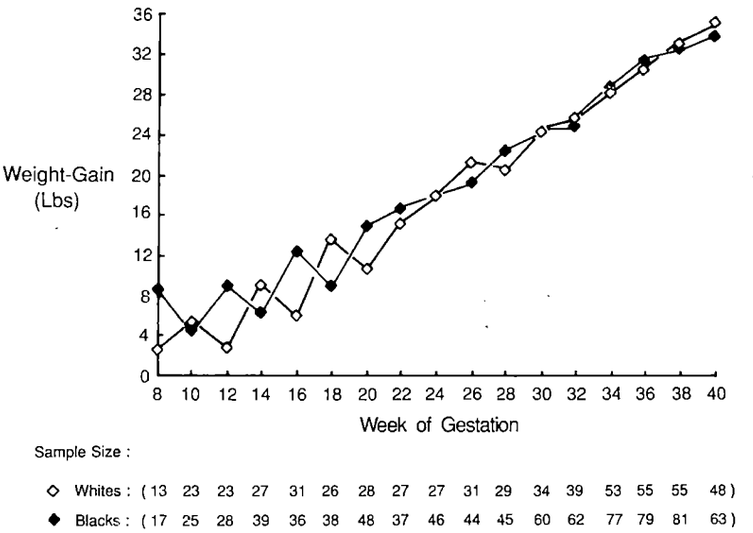


Fig. 1. Weight gain by gestational age in white women and black women delivered of singleton infants who weighed ≥3000 gm; Alameda County Low Birth Weight Study, Kaiser Permanente subset.

Table I. Cumulative weight gain by race of gestational age in women delivered of infants who weighed ≥3000 gm

	Black women (N = 101)			*White women (N = 61)*		
Gestational age (wk)	*No. of observations*	*Mean weight gain (lb)*	*SD of weight gain*	*No. of observations*	*Mean weight gain (lb)*	*SD of weight gain*
7-8	17	8.5	10.3	13	2.4	5.2
9-10	25	4.2	8.7	23	5.3	4.0
11-12	28	8.9	13.2	23	2.7	6.8
13-14	39	6.2	7.7	27	8.9	8.8
15-16	36	12.3	13.4	31	5.8	6.4
17-18	38	9.0	10.1	26	13.4	10.2
19-20	48	14.8	13.0	28	10.5	7.5
21-22	37	16.4	12.4	27	15.0	6.1
23-24	46	17.9	13.2	27	17.9	13.0
25-26	44	19.2	12.5	31	21.2	13.1
27-28	45	22.4	11.1	29	20.5	9.9
29-30	60	24.5	15.7	34	24.2	14.0
31-32	62	24.8	12.7	39	25.7	9.5
33-34	77	28.0	15.3	53	28.1	13.6
35-36	79	31.3	15.4	55	30.4	13.9
37-38	81	32.5	15.9	55	33.0	15.2
39-40	63	33.9	17.6	48	35.1	15.4

facilities, where standardized prenatal records are kept and >90% of women attend prenatal care in the first trimester. A single trained medical records abstractor recorded information on prepregnancy weight, the date of each prenatal visit, and maternal weight at each visit. Physicians' estimates of gestational age at the first prenatal visit were used to determine gestational age at each subsequent prenatal visit. Weight gain at each visit was calculated as the difference between weight at a given visit and prepregnancy weight. In the analysis, information on weight gain for all women who attended prenatal care in a given gestational interval was used to calculate the mean weight gain for that interval. If a woman attended prenatal care more than once in an interval, only weight gain at the first visit in the interval was used in the calculation of the mean.

The Student t test was used to assess the statistical significance of differences in weight gain. A probability value of 0.05 was considered statistically significant.

Results

Twenty-four women delivered of infants at a Kaiser Permanente facility were excluded because of inability to retrieve the medical record (n = 12), no prenatal care (n = 7), or prenatal care by a private physician (n = 5). There remained 56 black women and 63 white women delivered of low-birth-weight infants and 101 black women and 61 white women delivered of infants who weighed ≥3000 gm. Four women contributed no information to the analysis because an estimate of either gestational age or prepregnancy weight was missing.

In white women delivered of an infant who weighed ≥3000 gm, mean weight gain was 5.3 pounds at 9 to 10 weeks' gestation, 10.5 pounds at 11 to 20 weeks, 24.2 pounds at 21 to 30 weeks, and 35.1 pounds at 31 to 40 weeks (Table I). Mean weight gain in black women delivered of infants who weighed ≥3000 gm was 4.2 pounds at 9 to 10 weeks' gestation, 14.8 pounds at 11 to 20 weeks, 24.5 pounds at 21 to 30 weeks, and 33.9 pounds at 31 to 40 weeks. None of the differences in weight gain between black women and white women was statistically significant ($p > 0.05$). In both white and

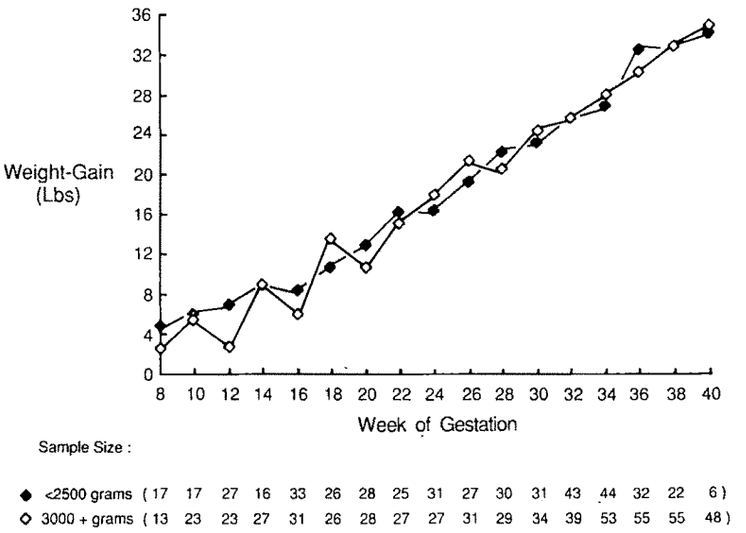


Fig. 2. Weight gain by gestational age in white women delivered of singleton infants who weighed ≥3000 gm, or ≤2500 gm; Alameda County Low Birth Weight Study, Kaiser Permanente subset.

black patients, the SD of weight gain was large at every gestational age. At 31 to 40 weeks' gestation, weight gain ranged from −8 pounds to 89 pounds in white subjects and 4 pounds to 99 pounds in black subjects.

The pattern of weight gain in both black and white women delivered of infants who weighed ≥3000 gm was linear after week 20 (Fig. 1). The rate of weight gain was linear at 1.06 pounds per week after 20 weeks' gestation. The rate of weight gain did not slow in the last 10 gestational weeks.

There was more interval-to-interval variation in average weight gain at earlier rather than at later gestational ages. The appearance of decreasing weight gain in the early gestational intervals is probably a result of the small number of observations in some of these intervals and to the high degree of variability of weight gain relative to the mean. By combining observations from white women and black women delivered of normal-weight infants, one can "smooth" the curve for the period of gestation from weeks 8 through 20. A regression line fitted to these combined data with the use of all data points yields an estimate of the rate of weight gain of 0.66 pounds per week for the interval from 8 through 20 weeks' gestation. Data on weight gain for gestations <7 weeks were so limited that mean weight gain was not calculated.

In white subjects, mean weight gain was essentially the same and not statistically significantly different ($p > 0.05$) at every gestational age in women delivered of low-birth-weight infants compared with those delivered of infants who weighed ≥3000 gm (Fig. 2). In black subjects, mean weight gain in women delivered of low-birth-weight infants was consistently lower than that in women delivered of infants who weighed ≥3000 gm after the 20 weeks' gestation (Fig. 3), although none of these differences was statistically significant ($p > 0.05$).

Comment

Total weight gain in women in our study population was higher than that in other U.S. populations reported in publications during the period 1955 through 1989 (Table II) except that of Abrams and Laros.[17] Total weight gain is higher in more recent studies, suggesting there is a secular trend of higher total weight during pregnancy. A secular trend of greater weight gain would explain the discrepancy between the results of previous studies and both our study and that of Abrams and Laros.[17] The published studies based on pregnancies that occurred in the 1950s and early 1960s encompassed an era when attempts were made to restrict weight gain during pregnancy, and the lower weight gains in these populations may be attributable to this now-abandoned policy. The nonrepresentative nature of previous studies, except that of Taffel,[16] limits the ability to draw a firm conclusion about time trends in weight gain.

A committee of the National Academy of Sciences[18] summarized published studies on weight gain by gestational age. Table III, derived from their report, presents our results along with data on weight gain by week of gestation from the only other studies of well-nourished women with uncomplicated pregnancies reported during 1949 through 1989. Weight gain in U.S. women was higher at every gestational age than that in the British study population. However, the two U.S.

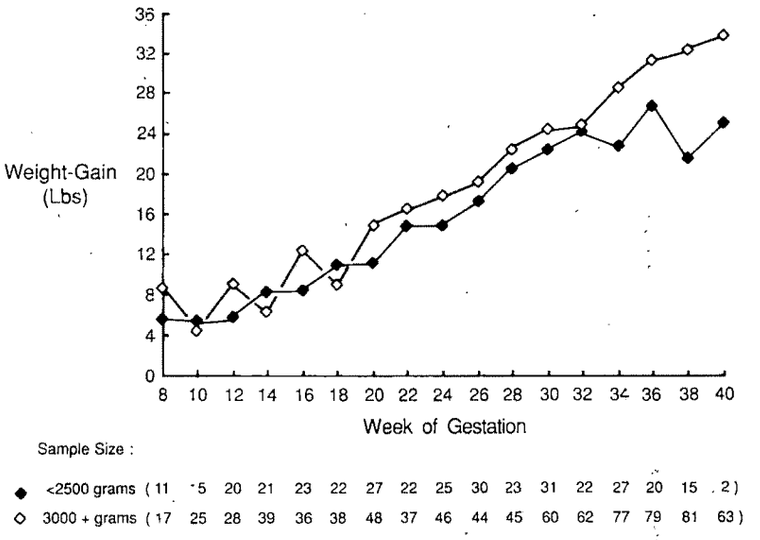


Fig. 3. Weight gain by gestational age in black women delivered of singleton infants who weighed ≥3000 gm, or ≤2500 gm; Alameda County Low Birth Weight Study, Kaiser Permanente subset.

Table II. Mean total weight gain during pregnancy for U.S. women as reported in studies published from 1950 to 1989

Study	*No. of women*	*Year*	*Race*	*Mean weight gain (lb)*	*Comment*
Eastman and Jackson[14]	25,154	1954-1961	—	21.8	39-42 wk
Niswander and Jackson[15]	5,755	1959-1966	White	21.7	singleton,
	6,012		Black	21.5	39-42 wk
Taffel[16]	Not reported*	1980	White	29.1	—
			Black	26.8	—
Abrams and Laros[17]	2,946	1980-1983	—	33.4	37+ wk
Petitti, Croughan-Minihane, and Hiatt	48	1987	White	35.1	singleton,
	63		Black	33.9	>3000 gm

*Data derived from the National Natality Survey and National Fetal Mortality Survey.

Table III. Cumulative weight gain (pounds) by gestational age for women with uncomplicated pregnancies reported in studies published during 1949 to 1989

			Weeks' gestation		
Study	*No.*	*Year*	*16*	*20*	*32*
Thomson and Billewicz[9]	2868	1949-1954	3.1	7.2	19.4
Brown et al.[1]	384	1979-1982	9.7	14.2	26.4
Petitti, Croughan-Minihane, and Hiatt	162	1987	9.3	13.2	25.1

studies are also more recent than the United Kingdom study. Weight gain at comparable gestational ages in the other U.S. study[1] is very similar to weight gain in our population at every gestational age.

We could not identify prior published studies that compared weight gain by gestational age in black women and white women. This is rather surprising because the existence of a 200 to 250 gm difference in birth weight between infants born to black women and white women is well recognized, and the question of differences in pattern of maternal weight gain as an explanation for the difference is an obvious one. In the present study, we found no large difference in mean weight gain between black women and white women delivered of infants who weighed 3000 gm.

Beginning at 20 weeks' gestation, weight gain in black mothers delivered of infants who weighed <2500 gm was less than that in the black women delivered of infants who weighed ≥3000 gm, although the difference was not statistically significant. Even if the difference were significant in a larger sample, the variability of weight gain and the small difference in weight gain between mothers delivered of low-birth-weight and normal-weight infants limits the usefulness of weight gain patterns as a screening tool for identification of women at risk of being delivered of low-birth-weight infants.

Our study has some important limitations. First, the size of the study group is small. This limits the statistical power to find differences in weight gain, although the high variability of weight gain would not be reduced in a larger sample. The small sample size makes examination of modifiers of weight gain, such as prepregnancy weight, unfruitful. Second, we relied on the date of the last menstrual period as recorded at the first routine prenatal visit to estimate gestational age at subsequent prenatal visits. Estimates of gestational age derived in this way are known to be imperfect,[19] although they seem reasonable for term infants, who comprise the majority of our study population. Third, we used the information on weight that was recorded as part of routine prenatal care. Lack of standardization of procedures to record weight and of the timing of prenatal visits may have biased our findings in ways that are unknown.

Several advantages exist for our study population and our methods. The population was largely middle class. Few women experienced prenatal complications, and all women attended regularly scheduled prenatal care appointments. In addition, there was little or no missing information on the variables of interest.

Data from this study were easily obtainable from routine prenatal care records. Further delineation of factors that influenced the pattern of weight gain and identification of the association of pattern of weight gain and pregnancy outcome with routine prenatal care records may be a fruitful area for further research.

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The effects of hormone replacement therapy in normal postmenopausal women: Measurements of Doppler-derived parameters of aortic flow

Amos Pines, MD,[a, d] Enrique Z. Fisman, MD,[c] Yoram Levo, MD,[a]
Mordechai Averbuch, MD,[a, d] Arie Lidor, MD,[b, d] Yaacov Drory, MD,[c]
Ariel Finkelstein, MD,[a, d] Miriam Hetman-Peri, MD,[b, d] Menachem Moshkowitz, MD,[a, d]
Efraim Ben-Ari, PhD,[c] and Daniel Ayalon, MD[b, d]
Tel Aviv, Israel

In this study the effects of hormone replacement therapy on cardiac function in healthy postmenopausal women were evaluated by Doppler echocardiography that was performed before (T1) and 2.5 months after the initiation of hormone replacement therapy (T2) in the peak estrogenic phase. The following parameters of aortic flow were measured: peak flow velocity, acceleration time, and ejection time. Additional parameters were calculated: flow velocity integral and mean acceleration. The study group included 24 postmenopausal women aged 43 to 60 years (mean 51.6 years). The control group consisted of 19 postmenopausal women aged 46 to 60 years (mean 53.5 years) who were not receiving hormone replacement therapy and who underwent the same evaluation. There were no changes in all Doppler parameters between T1 and T2 in the control group. However, in the study group there were significant increases in peak flow velocity (108.3 ± 16.7 cm/sec at T1 vs 123 ± 20.7 cm/sec at T2; $p = 0.002$), flow velocity integral (17.7 ± 3.9 vs 21.5 ± 4.7 cm; $p = 0.0003$), mean acceleration (11.5 ± 1.9 vs 13.1 ± 2.6 m/sec/sec; $p = 0.001$), and ejection time (324 ± 37.6 vs 348.8 ± 40.7 msec; $p = 0.002$). There was no change in acceleration time (94.8 ± 6.6 vs 95 ± 10.9 msec). These results demonstrate that estrogens increase both stroke volume and flow acceleration. The latter probably reflects a combination of enhanced inotropism and vasodilatation. We assume that the cardioprotective effect of hormone replacement therapy in postmenopausal women may be due not only to changes in lipid profile but also to direct effects of estrogens on central and peripheral hemodynamic parameters. (Am J Obstet Gynecol 1991;164:806-12.)

Key words: Menopause, hormone replacement therapy, aortic blood flow, Doppler echocardiography

During the past 10 years there has been a growing interest in estrogen replacement therapy for postmenopausal women.[1, 2] The effects of estrogen replacement therapy in this population have been studied extensively, and changes that have been induced by estrogens in the genitourinary system, bone, and liver and in major metabolic pathways have been well documented. It has been known since the beginning of this century that estrogens also exert hemodynamic changes through several different mechanisms.[3, 4] Plasma volume, stroke volume, and cardiac output were found to increase, and peripheral resistance was found to decrease in women who were taking oral contraceptives, during pregnancy, or during the normal menstrual cycle, correlating with the elevation of estrogen levels. However, information on hemodynamic effects of estrogen replacement therapy in postmenopausal women is scarce.[5]

Several reports have discussed the nonspecific electrocardiographic ST-T changes that are induced by estrogens, which resemble those that are induced by digoxin and which in fact provide indirect evidence for the influence of estrogens on the myocardium.[6] Evidence for a direct effect of estrogens on heart muscle was obtained in animal studies. These included the identification of physiologically functioning estrogen receptors in the heart and aorta[7, 8] and changes in myosin-adenosinetriphosphatase and lysosomal enzyme activities after estrogen deprivation or supplementation.[9] In view of these data in humans and animals, we decided to evaluate several Doppler-derived

From the Department of Medicine T[a] and the Tirnsit Institute for Reproductive Endocrinology,[b] Ichilov Hospital, Tel Aviv Sourasky Medical Center, the Cardiac Rehabilitation Institute, Sheba Medical Center, Tel Hashomer,[c] affiliated with the Sackler Faculty of Medicine, Tel Aviv University, and the Menopause Clinic of the Maccabi Medical Care and Health Fund, Tel Aviv.[d]
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Reprint requests: Amos Pines, MD, Department of Medicine T, Ichilov Hospital, 6 Weizman St., Tel-Aviv, Israel 64239.
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parameters in postmenopausal women who were receiving hormone replacement therapy.

Patients and methods

During a 2-month period, all women who were referred for consultation at the Menopause Clinic were asked to participate in the study. Postmenopausal state was established by serum estradiol levels of less than 20 pg/ml and follicle-stimulating hormone levels of at least 30 mIU/ml. Exclusion criteria were: age over 60 years, hypertension, diabetes mellitus (or any other endocrinopathy) or treatment with medications that might affect cardiac function. Also excluded were patients with heart disease (on the basis of physical examination, past history, and electrocardiographic results). The remaining women were divided into two groups according to the decision of whether or not to recommend hormone substitution with a sequential regimen of combined estrogen-progesterone treatment. The study group, which was receiving hormone replacement therapy, was evaluated by Doppler echocardiography before treatment (T1) and in the middle of the third cycle of hormone replacement therapy (T2), in the peak estrogenic phase and at least 2 weeks after treatment with progesterone of the previous hormonal cycle. The control group, which was not receiving hormone replacement therapy, also had two sets of Doppler examinations at similar time intervals.

Doppler technique. Examinations were performed with a wide-angle phased-array Hewlett Packard Sonos 500 imaging system (Hewlett Packard Co., Medical Products Gr., Andover, Mass.) with a pulsed Doppler transducer. Doppler studies were performed from the suprasternal notch, and the ascending aorta was interrogated. The sample volume depth was gradually adjusted until the optimal flow pattern that showed the highest velocity with the least spectral dispersion was found. The axial size of the sample was 5 mm.

Sample size, spectral depth, and position in the vessel remained constant throughout the study. Before the examination of each patient, the Doppler echocardiography operator familiarized himself or herself with the optimal window and angulation for the subject and also made the necessary adjustments in the equipment. Measurements were taken from frozen images and averaged 5 beats for each examination.

Doppler examinations were done at a speed of 100 mm/sec and taped on a commercial Panasonic AG-6200 videotape recorder (Matsushita Electric Corp. of America, Seacaucus, N.J.). The images were replayed for analysis in real-time, slow-motion, and freeze-frame modes, and they were reviewed independently by two observers who were unaware of the subjects' clinical data.

Doppler measurements. Peak flow velocity, expressed in centimeters per second, was defined as the midpoint of the upper portion of the Doppler signal at the time of maximal velocity. Ejection time, expressed in milliseconds, was defined as time from onset until end of systolic flow; both points were determined by the time at which the Doppler signal crossed the zero-flow line. Acceleration time, expressed in milliseconds, was defined as the time from onset of flow to peak velocity point. Mean acceleration was calculated as peak flow velocity/acceleration time and expressed in meters per second per second. The flow velocity integral, which represents the area under the systolic portion of the velocity signal, reflects the forward distance that is traveled by the erythrocytes during a given systole. After delineation of the contour of the signal with the joystick, the flow velocity integral was internally derived by the system on the basis of planimetric integration of the pixels that were included in the flow envelope, and it was expressed in centimeters.

Statistical analysis. Values were expressed as mean ± standard deviation. The percent change in Doppler-derived parameters of aortic flow was calculated as (T2 value − T1 value)/T1 value. For comparisons between T1 and T2 data within the groups we used Student's two-tailed t test for paired observations, whereas for intergroup comparisons we used the unpaired t test. A p value of <0.05 was considered significant.

Results

The study group included 24 women, aged 43 to 60 years (mean 51.6 ± 3.9 years), 21 (87.5%) of whom had natural menopause. The mean time from menopause was 40 ± 42 months. The control group consisted of 19 women, aged 46 to 60 years (mean 53.5 ± 4.4 years), 15 (78.5%) of whom had natural menopause. Time from menopause was 52 ± 48 months. The average weight for the control group exceeded that of the study group at the baseline value (67.9 ± 10.4 vs 61.1 ± 10 kg, $p = 0.04$); however, weight did not change significantly in both groups from T1 to T2. Blood pressure and heart rate were similar in both groups at T1 and T2. Hormone replacement therapy consisted of conjugated estrogens 0.625 mg for 12 days combined with medroxyprogesterone acetate 5 mg over the following 13 days (Premaril plus, Dexxon, Herzliya, Israel) in 17 women and skin patches of estradiol 4 mg, which were applied twice a week (Estraderm Transdermal System 50, Ciba Pharmaceutical Co., Summit, N.J.) combined with oral medroxyprogesterone acetate 5 mg (Aragest, Dexxon) on days 13 to 22 of each cycle in seven women.

Doppler measurements. Diagnostic quality suprasternal Doppler images were obtained in all subjects. The intraobserver variability was 3.2% for peak flow velocity, 2.2% for ejection time, and 3.0% for acceleration time. The interobserver variability was 3.5% for peak flow velocity, 2.4% for ejection time, and 2.9% for

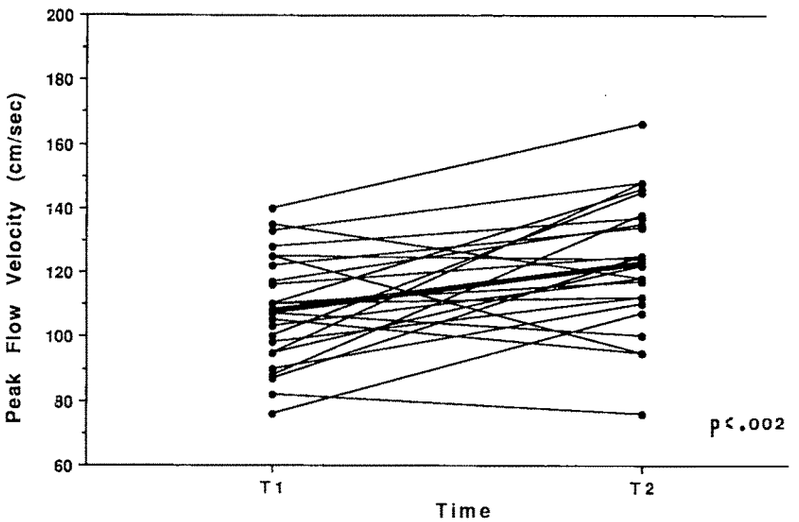


Fig. 1. Peak flow velocity values before (T1) and after (T2) hormone replacement therapy ($N = 24$).

Table I. Comparison of parameters of aortic flow before (T1) and after (T2) hormone replacement therapy in postmenopausal women

Parameters	*PMW with HRT*	*PMW without HRT*	*p Value*
PFV1 (cm/sec)	108.3 ± 16.7	120.2 ± 18.8	0.02
PFV2 (cm/sec)	123.0 ± 20.7	124.2 ± 19.6	
p Value	0.002	NS	
Percent change*	17.4 ± 20	4.4 ± 15.3	0.02
FVI1 (cm)	17.7 ± 3.9	19.4 ± 3.5	0.067
FVI2 (cm)	21.5 ± 4.7	20.1 ± 4.5	
p Value	0.0003	NS	
Percent change	25.8 ± 23.9	3.9 ± 14.6	0.001
AT1 (msec)	94.8 ± 6.6	99.2 ± 8.2	0.04
AT2 (msec)	95.0 ± 10.9	99.5 ± 7.8	
p Value	NS	NS	
Percent change	0.6 ± 7.5	0.5 ± 5.7	NS
ET1 (msec)	324.0 ± 37.6	321.1 ± 33.5	NS
ET2 (msec)	348.8 ± 40.7	321.8 ± 37.8	
p Value	0.002	NS	
Percent change	8.2 ± 12	1.6 ± 5.7	0.03
MA1 (m/sec^2)	11.5 ± 1.9	12.2 ± 2.1	NS
MA2 (m/sec^2)	13.1 ± 2.6	12.5 ± 2.2	
p Value	0.001	NS	
Percent change	15.4 ± 21	4.1 ± 16.9	0.06

PMW, Postmenopausal women; *HRT*, hormone replacement therapy; *PFV*, peak flow velocity; *NS*, not significant; *FVI*, flow velocity integral; *AT*, acceleration time; *ET*, ejection time; *MA*, mean accelerations.

*Percent change = value of (T2 − T1)/T1 × 100.

acceleration time. None of these differences were significant. The aortic valve dimensions remained unchanged at T1 and T2.

The mean measured values of peak flow velocity and acceleration time at T1 were higher for the control group than for the study group (120.2 vs 108.3 cm/sec, $p = 0.02$; 99.2 vs 94.8 msec, $p = 0.04$) (Table I). There was no difference between the groups regarding ejection time (324 vs 321.1 msec). The calculated flow velocity integrals of the control group at baseline were thus higher than those of the study group, although they did not reach statistical significance (19.4 vs 17.7 cm, $p = 0.067$). These differences in T1 could reflect the greater weight of women in the control group, since stroke volume is positively correlated with body weight.

The study group showed very significant increases ($p < 0.001$) in peak flow velocity, flow velocity integral, ejection time, and mean acceleration from T1 to T2 (Table I and Figs. 1 to 6), but acceleration time did not change. Peak flow velocity and flow velocity integral decreased in five women only, ejection time was reduced only in three, and acceleration time was reduced in seven. In spite of more prominent intragroup variations in all parameters in the control group, there was no change in any of the parameters from T1 to T2 for that group as a whole.

In a comparison of the percent change of the above variables from T1 to T2, both groups showed similar results (Table I). In the study group the peak flow velocity increased by a mean of 17.4%, the flow velocity integral by 25.8%, ejection time by 8.3%, and mean acceleration by 15.4%. The corresponding mean changes in the control group were 4.4% ($p = 0.02$), 3.9% ($p = 0.001$), 1.6% ($p = 0.03$), and 4.1% ($p = 0.066$). Acceleration time changed by only 0.6% in the study group and by only 0.5% in the control group. A review of the echocardiographic results in women who

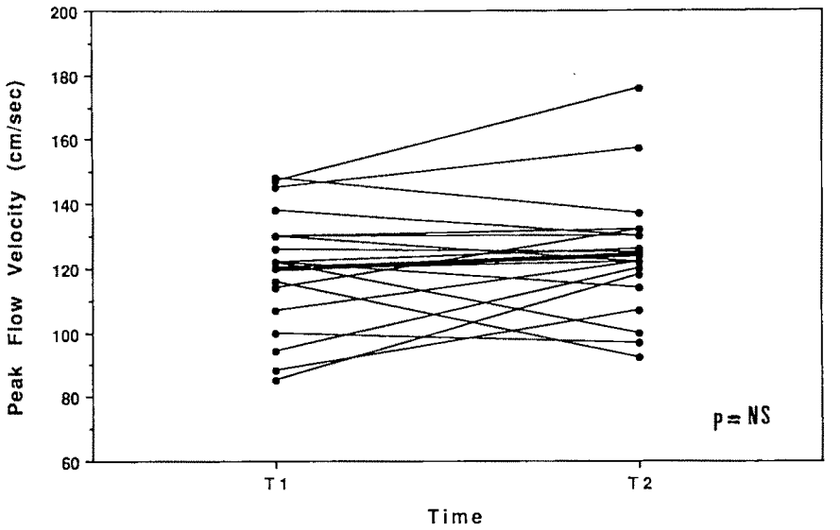


Fig. 2. Peak flow velocity values in control group (N = 19) at beginning (T1) and termination (T2) of study.

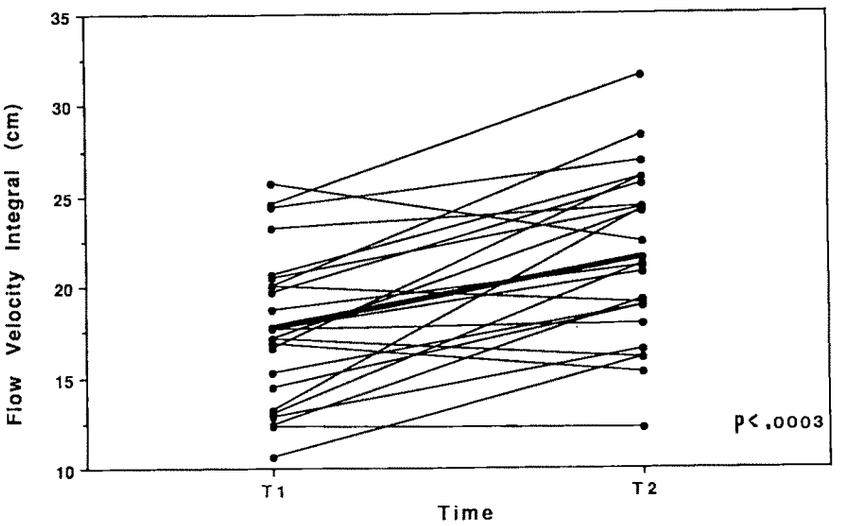


Fig. 3. Flow velocity integral before (T1) and after (T2) hormone replacement therapy (N = 24).

were taking Premaril plus versus women who were taking Estraderm Transdermal System 50 showed that there was no differences between the groups in all of the parameters that were tested.

Comment

The incidence of coronary artery disease in premenopausal women is lower when compared with age-matched men but increases rapidly after menopause. This observation suggested that estrogens have cardioprotective properties.[2, 10] Long-term epidemiologic studies of postmenopausal women who were receiving estrogen replacement therapy revealed a significant decrease in cardiovascular morbidity and mortality rates.[10-12] The cardioprotective effect of estrogen replacement therapy was attributed mainly to beneficial alterations in lipid profile,[13] but other pathophysiologic mechanisms probably exert an additional protective effect. In the present study we examined, by Doppler echocardiography, the possible effects of hormone replacement therapy on aortic flow.

Our main observations were highly significant increases in peak flow velocity, flow velocity integral, and mean acceleration after 2.5 months of hormone replacement therapy. Some of these findings are in accord with previously published echocardiographic data on

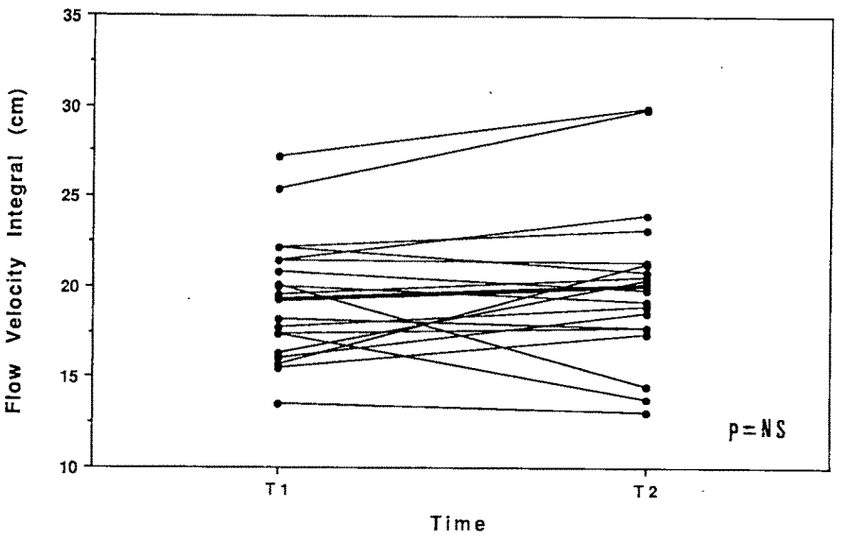


Fig. 4. Flow velocity integral values in control group (N = 19) at beginning (T1) and termination (T2) of study.

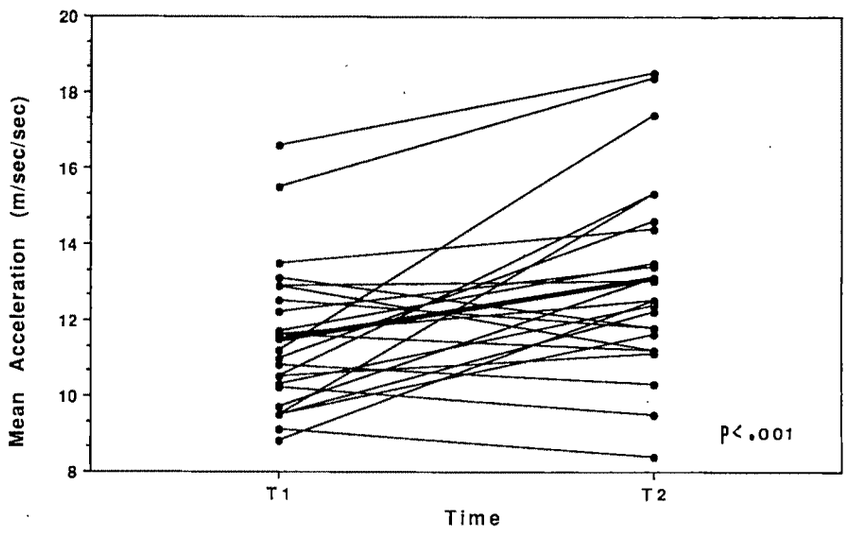


Fig. 5. Mean acceleration before (T1) and after (T2) hormone replacement therapy (N = 24).

women in hyperestrogenic states,[14-18] although one must note that hormone levels in women who are receiving hormone replacement therapy are very low compared with those in any of the following conditions: Mashini et al.,[14] with the use of the M-mode technique to monitor stroke volume and ejection fraction throughout pregnancy, found an increase in cardiac output, which they attributed to a higher heart rate and a lower systemic vascular resistance. Robson et al.[15] compared cardiac output as determined by the Fick technique with Doppler measurements of three valve flows in pregnant and nonpregnant women. They found that measurements at the aortic valve provided the closest concordance with cardiac output as determined by the Fick method. La Sala et al.[16] investigated the cardiovascular adaptation to rapid increase of estrogen levels during multiple follicular stimulation, late luteal phase, or early pregnancy. With two-dimensional and Doppler echocardiography, they demonstrated an increase in cardiac index and in left ventricular end-systolic and end-diastolic dimensions and a decrease in systemic vascular resistance. They also showed that these hemodynamic modifications occurred within less than 2 weeks after the acute changes in estrogen levels. Vielle

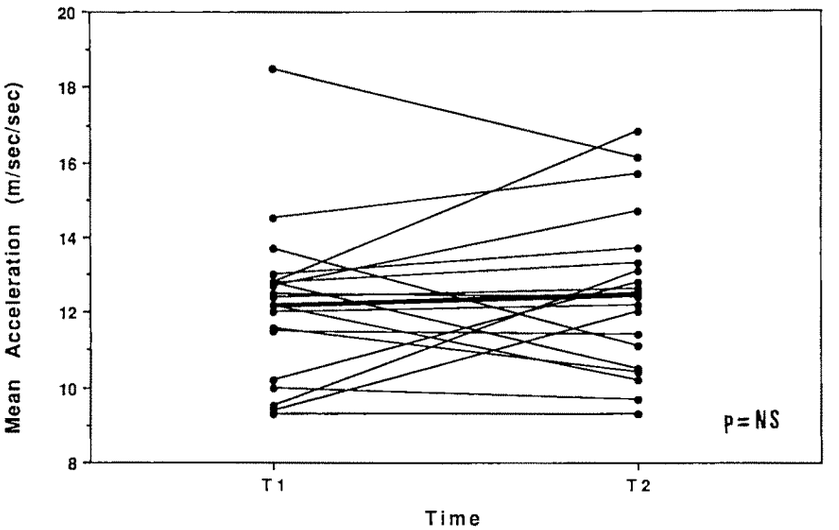


Fig. 6. Mean acceleration values in control group (N = 19) at beginning (T1) and termination (T2) of study.

et al.[17] evaluated changes during ovulation induction by M-mode echocardiography. They found significant increases in left ventricular diastolic dimension, stroke volume, and cardiac index, which were accompanied by a concomitant decrease in systemic vascular resistance. These changes were evident within a week and paralleled serum estrogen levels. Kessler et al.[18] compared echocardiographic parameters of left ventricular size and function in young women who were and who were not taking oral contraceptives. No differences between these groups were observed in any of the measured parameters.

The increase in flow velocity integral, which parallels an increase in stroke volume (stroke volume = flow velocity integral × aortic valve area), was therefore expected in our treated patients. The observed increase in mean acceleration deserves special attention. Acceleration is not influenced by preload and only marginally affected by heart rate.[19] In fact, it has been shown that flow acceleration is a useful index of myocardial contractility, which correlates well with ejection fraction.[20] It is also very sensitive to changes in the contractile state.[19] Hence the increase in mean acceleration might reflect a positive inotropic effect of estrogens. In fact, such an effect could be expected on the basis of the structural similarity of estrogens and digoxin. Furthermore, animal studies[21] have suggested that estrogens may upregulate the density of both muscarinic and β-adrenergic cardiac receptors and thus contribute to enhanced contractility. Further studies are obviously needed to establish a direct inotropic effect of estrogens in human and to evaluate the possible role of such an effect on cardiovascular morbidity. In conclusion, measurement of parameters of aortic flow by Doppler echocardiography showed that hormone replacement therapy in postmenopausal women is associated with an increase in stroke volume and flow acceleration. Since menopause carries a potentially higher risk for cardiovascular morbidity and since hormone replacement therapy is now strongly recommended for the majority of postmenopausal women, it is crucial to further evaluate all potential effects of such therapy on the cardiovascular system.

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Decreased levels of polyunsaturated fatty acids in preeclampsia

Yuping Wang, MD, Helen H. Kay, MD, and Allen P. Killam, MD
Durham, North Carolina

Plasma levels of polyunsaturated fatty acids in the *n*-3 and *n*-6 classes, which include linoleic, linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids, were quantified with high-performance liquid chromatography in nonpregnant volunteers and in patients with normal pregnancies or preeclampsia at term. The total polyunsaturated fatty acid levels were not significantly different between nonpregnant and normal pregnant patients but was significantly lower in the preeclamptic patients compared with normal pregnant patients. This decreased level could represent altered fatty acid metabolism or altered storage and mobilization from lipid pools. Compared with nonpregnant patients, normal pregnant patients had significantly higher levels of eicosapentaenoic and docosahexaenoic acid. This may reflect normal physiologic changes in pregnancy, and the decreased level of eicosapentaenoic acid seen in preeclamptic patients may play a significant role in the pathophysiology of preeclampsia. (Am J Obstet Gynecol 1991;164:812-8.)

Key words: Preeclampsia, polyunsaturated fatty acids, high performance liquid chromatography

The prostanoids thromboxane and prostacyclin have been of special obstetric interest over the past few years, since preeclampsia may be related to an imbalance in prostacyclin and thromboxane production.[1, 2] Prostacyclin is a potent vasodilator and thromboxane is a potent vasoconstrictor. The importance of these substances extends our interest to the entire group of polyunsaturated fatty acids, some of which are precursors to these prostanoids (Fig. 1). The two main classes of polyunsaturated fatty acids that have been the focus of clinical attention are the *n*-6 and *n*-3 classes. Linoleic acid is the main precursor of the *n*-6 class, and linolenic acid is the precursor of the *n*-3 class. Both these acids are essential fatty acids because humans cannot synthesize them and therefore must ingest them in the diet. Linoleic acid is the immediate precursor to arachidonic acid which is converted by cyclooxygenase to the prostanoids, which include prostacyclin and thromboxane. Arachidonic acid is also converted by lipooxygenase to leukotrienes. Linolenic acid is the precursor

From the Department of Obstetrics and Gynecology, Duke University Medical Center.
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Reprint requests: Helen H. Kay, MD, P.O. Box 3373, DUMC, Durham, NC 27710.
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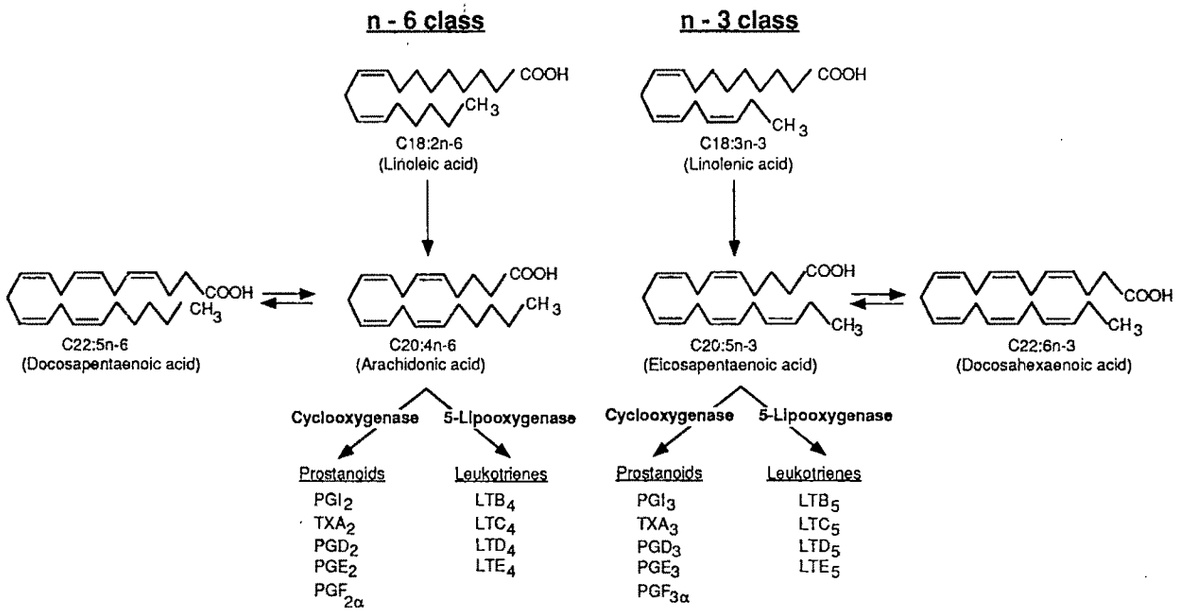


Fig. 1. Metabolic pathways of *n*-3 and *n*-6 polyunsaturated fatty acids.

of eicosapentaenoic acid, which is then reversibly converted to docosahexaenoic acid. Eicosapentaenoic acid is also metabolized by cyclooxygenase and lipooxygenase to different prostanoids and leukotrienes with biologic properties different from those derived from arachidonic acid.[3]

Other investigators have previously noted a relationship of polyunsaturated fatty acids to the control of hypertension. Bang et al.[4] reported in the mid 1970s that Eskimos in Greenland who had a low mortality from myocardial infarction ate a diet high in polyunsaturated fats. Their diet, which primarily consists of fish, is rich in *n*-3 polyunsaturated fatty acids. Subsequently, several randomized, placebo-controlled clinical studies of hypertensive patients have demonstrated a beneficial effect of fish oil in reducing blood pressure, suggesting that the *n*-3 class of polyunsaturated fatty acids, particularly eicosapentaenoic acid, plays an important role in regulating vascular disease.[5] It is thought that a diet high in these fatty acids can decrease effective thromboxane and increase effective prostacyclin production and therefore correct an underlying imbalance between these agents. These fatty acids are capable of reducing triglyceridemia, cholesterolemia, and platelet aggregation, all of which may lower the incidence of atherosclerotic disease.[6] Additionally, these fatty acids can decrease blood viscosity, increase erythrocyte deformity, and increase antithrombin levels.

The relationship between the *n*-3 and *n*-6 classes of fatty acids has not been well studied in pregnancy. Eicosapentaenoic acid is capable of reducing prostacyclin and thromboxane A_2, because its derivatives, prostaglandin I_3 and thromboxane A_3, can compete with arachidonic acid for cyclooxygenase activity.[7] An interrelationship between *n*-3 and *n*-6 fatty acids may represent normal regulatory mechanisms for the maintenance of vascular tone. The effects of arachidonic acid–derived prostanoids in preeclampsia may be regulated by prostanoids derived from eicosapentaenoic acid in the *n*-3 class of fatty acids. The purpose of this study was to further elucidate the relationships between these two classes of polyunsaturated fatty acids by investigating the levels of *n*-6 (linoleic acid and arachidonic acid) and *n*-3 (linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid) fatty acids in nonpregnant, normal pregnant, and preeclamptic patients.

Material and methods

Blood samples were obtained from nonfasting volunteers and patients after they had given informed consent. Subjects were all between 20 and 40 years of age and included 11 normal pregnant patients, 9 preeclamptic patients, and 10 nonpregnant female volunteers as controls. Preeclampsia was defined as blood pressure ≥140/90 mm Hg on two separate readings at least 6 hours apart, proteinuria, and edema. All normal and preeclamptic patients were at term, and none had been on a regimen of aspirin therapy before entering the study. The control group subjects were not using oral contraceptives. Blood samples were drawn into ethylenediaminetetraacetic acid Vacutainer blood-collecting tubes (Becton-Dickinson, Rutherford, N.J.). The plasma was separated by centrifugation at

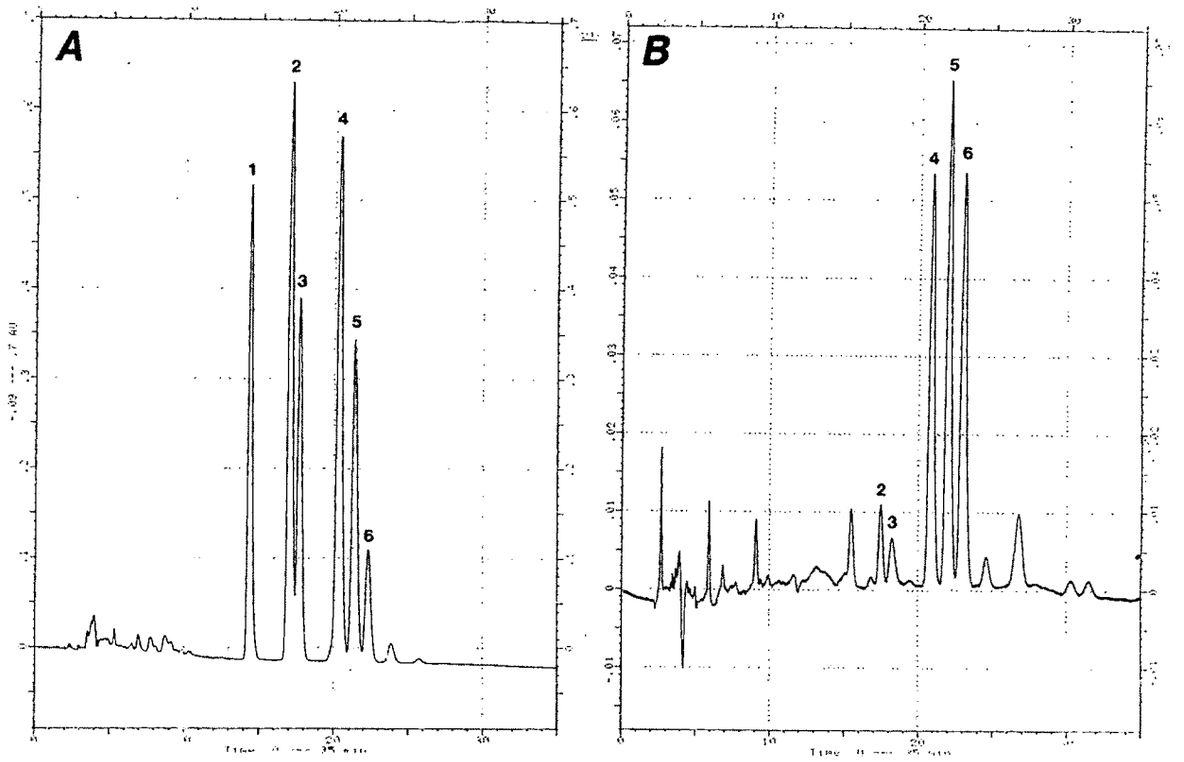


Fig. 2. A, Chromatogram of six polyunsaturated fatty acid standards. Only five were pertinent to our current study. Note clean separation of peaks. *1,* Octadecatetraenoic acid; *2,* eicosapentaenoic acid; *3,* linolenic acid; *4,* docosahexaenoic acid; *5,* arachidonic acid; *6,* linoleic acid. **B,** Chromatogram of plasma sample from study subject showing five fatty acids of interest in current study. *2,* Eicosapentaenoic acid; *3,* linolenic acid; *4,* docosahexaenoic acid; *5,* arachidonic acid; *6,* linoleic acid.

1000 rpm for 10 minutes and stored at −20° C until analysis was performed.

Fatty acids were analyzed by high-performance liquid chromatography carried out on a gradient system (Waters model 600E, Millipore Corp., Milford, Mass.) equipped with a guard column PR 8 or PR 4 (Brownlee Lab, Inc., Santa Clara, Calif.) and reversed phase column of octyl (particle size 5 μm, 10.0 mm × 2.5 cm inner diameter, Beckman Instruments, Inc., Fullerton, Calif.) linked in series. The separation of the methyl ester derived fatty acid mixture was carried out using methanol/water (90:10, vol/vol) as a mobile phase at a flow-rate of 3 ml/min. The eluent was monitored by a photodiode array detector (model 990, Water Dynamics). The wavelength was set at 210 nm. The liquid chromatography system was linked with a computer (Power Mate II, NEC, Tokyo).

Standards consisting of linoleic acid methyl ester, arachidonic acid methyl ester, linolenic acid methyl ester, 5,8,11,17-eicosapentaenoic acid methyl ester, and *cis*-4,7,10,13,16,19-docosahexaenoic acid ester were purchased from Sigma (St. Louis). No standard was available for docosapentaenoic acid; therefore it was not assayed. Acetyl chloride, methanol, and potassium carbonate anhydrous were purchased from Fisher Scientific Company (N.J.). Methyl alcohol of high-performance liquid chromatography reagent grade was obtained from Mallinckrodt (Paris, Ky.). All chemicals and solvents were of analytic reagent grade. Deionized and distilled water was used.

Fatty acids were measured in their methyl ester form. The methylation method was described by Lepage and Roy.[8] Briefly, 150 μl plasma was added to 5 ml of methanol/acetyl chloride (50:1, vol/vol), thoroughly mixed, and left at room temperature for 45 minutes. The methylation reaction was stopped by the slow addition of 3 ml of 6% potassium carbonate solution to the mixture. After 5 minutes, 1 ml of hexane was added and the mixture was vigorously shaken. The hexane layer, which contained the methylated fatty acids, was separated and dried under nitrogen. The residue was dissolved in acetonitrile for lipid analysis of high-performance liquid chromatography. A typical chromatogram obtained with a mixture of six polyunsaturated fatty acid standards is shown in Fig. 2, *A*, and one of a plasma sample is shown in Fig. 2, *B*. The

Table I. Plasma polyunsaturated fatty acid levels

	Nonpregnant (n = 10) (mg/L, mean ± SE)	*Normal pregnant (n = 11) (mg/L, mean ± SE)*	*Preeclampsia (n = 9) (mg/L, mean ± SE)*
Total polyunsaturated fatty acids	79.51 ± 3.47	90.60 ± 6.68 ($p < 0.05$)*	67.42 ± 3.88
Linoleic acid (18:2 *n*-6)	60.79 ± 2.28	62.93 ± 4.69 ($p < 0.01$)*	45.98 ± 2.80
Arachidonic acid (20:4 *n*-6)	10.99 ± 1.01	12.81 ± 0.87	11.44 ± 1.00
Linolenic acid (18:3 *n*-3)	1.88 ± 0.17	3.68 ± 0.99 ($p < 0.05$)*	1.11 ± 0.25
Eicosapentaenoic acid (20:5 *n*-3)	0.26 ± 0.04 ($p < 0.05$)†	1.08 ± 0.33 ($p < 0.05$)*	0.11 ± 0.11
Docosahexaenoic acid (22:6 *n*-3)	5.58 ± 0.60 ($p < 0.01$)†	10.40 ± 0.94	8.94 ± 0.69

*Normal pregnant versus preeclampsia.
†Nonpregnant versus pregnant.

individual fatty acids yielded single discrete peaks in the chromatogram. The methyl ester derivatives of the five plasma polyunsaturated fatty acids of interest could be separated within 30 minutes. All peaks of the polyunsaturated fatty acids in the methyl ester form were identified on the basis of their retention times and wavelengths of the eluents in comparison with the standard compounds.

Standard curves derived from plotting peak integrals were prepared for each fatty acid, and linearity was established within the lower and upper limits of detection. The correlation coefficients of the standard curves were: linoleic acid, 0.999; arachidonic acid, 1.000; linolenic acid, 0.999; eicosapentaenoic acid, 0.995; and docosahexaenoic acid, 0.999: To test our recovery of the fatty acids, varying amounts of free arachidonic acid were added to five plasma samples before methylation and analysis with high-performance liquid chromatography, as described above. Recovery of the added arachidonic acid was 98%.

Since levels of fatty acid precursors and derivatives might be influenced by food intake, two nonpregnant and two normal pregnant volunteers were tested while fasting and 2 hours after eating.

Statistical analysis comparing mean levels between nonpregnant and normal pregnant patients and between normal pregnant and preeclamptic patients was performed with the Student *t* test. A p value <0.05 was considered to be significant. Regression analysis was used to establish standard curves.

Results

A comparison of the mean serum concentrations of polyunsaturated fatty acids between nonpregnant and normal pregnant subjects and between normal pregnant and preeclamptic patients is presented in Table I. The total polyunsaturated fatty acids in the normal pregnant group was not significantly higher than in the nonpregnant patients but was significantly higher than in preeclamptic patients ($p < 0.05$). In the *n*-6 group of fatty acids, there was a significant difference between the plasma levels of linoleic acid in normal pregnancy compared to preeclampsia ($p < 0.01$), but there was no difference between normal pregnant patients compared with nonpregnant individuals. There were no significant differences in the arachidonic acid levels among any groups.

In the *n*-3 group of fatty acids, a significant difference was noted between the levels of linolenic acid in normal pregnant and preeclamptic patients ($p < 0.05$), but there was no statistical difference between the nonpregnant and normal pregnant patients. There were significant increases of eicosapentaenoic acid and docosahexaenoic acid in normal pregnant patients compared with nonpregnant patients. Although eicosapentaenoic acid was significantly lower in the preeclamptic patients than in the normal pregnant patients, there was no significant difference in docosahexaenoic acid levels between the two groups.

No statistical differences were noted in the five polyunsaturated fatty acid levels between fasting and nonfasting states in both nonpregnant and normal pregnant subjects (Table II).

Comment

Our current study is the first to report levels for several different polyunsaturated fatty acids in normal pregnancy and in preeclampsia. We found that the total level of the five plasma polyunsaturated fatty acids we studied were decreased in the preeclamptic patients compared with normal pregnant patients, but there was no difference between the levels in nonpregnant and normal pregnant patients. There is very little data, if any, in the published literature on plasma levels and

Table II. Fasting and postprandial polyunsaturated fatty acid levels

	Nonpregnant (mg/L)		*Normal pregnant* (mg/L)*	
	Subject 1	*Subject 2*	*Subject 3*	*Subject 4*
Linoleic acid (18:2, *n*-6)				
Fasting	54.41	60.20	65.27	69.33
Postprandial	53.92	65.22	64.16	67.66
Arachidonic acid (20:4, *n*-6)				
Fasting	5.53	8.28	24.97	22.25
Postprandial	5.95	8.42	26.90	23.65
Linolenic acid (18:3, *n*-3)				
Fasting	1.59	2.95	1.35	1.77
Postprandial	1.46	2.96	1.23	1.23
Eicosapentaenoic acid (20:5, *n*-3)				
Fasting	0.17	0.42	0.24	0.31
Postprandial	0.16	0.53	0.22	0.32
Docosahexaenoic acid (22:6, *n*-3)				
Fasting	3.53	5.43	17.27	15.87
Postprandial	3.90	5.26	16.99	17.15

*Normal pregnant volunteers were in the second trimester of pregnancy and therefore levels may differ from those observed in Table I in individuals at term.

clinical significance of the *n*-3 and *n*-6 classes of polyunsaturated fatty acids in normal or preeclamptic patients. Ogburn et al.[9] reported arachidonic acid levels that were higher than ours, but this most likely reflects the difference in method. One other group[10] reported that preeclamptic patients demonstrated an increased ratio of a diene conjugated isomer of linoleic acid to its nonisomerized form, as measured in our study. The fact that this ratio was increased suggests that the nonisomerized form of linoleic acid is decreased in preeclampsia, which is directly consistent with our current findings.

The lower level of plasma fatty acids seen in preeclamptic patients is not completely understood. A dietary deficiency has been suggested by investigators who noticed a higher incidence of preeclampsia in women from lower socioeconomic classes who had poor nutritional status. Another explanation may be a decreased fatty acid absorption from the gastrointestinal tract. Both of these possibilities are less likely because the body contains a large pool of essential fatty acids, and clinical deficiency states are rare in adults.[11]

Another more likely explanation for the reduced levels of plasma fatty acids may be altered metabolism in preeclamptic patients, resulting in more rapid conversion of linoleic and linolenic acids to their metabolites. The relatively low level of eicosapentaenoic acid suggests that it may also be more quickly metabolized to prostanoids and leukotrienes via the cyclooxygenase/lipooxygenase pathways, or to docosahexaenoic acid. Additional studies on both the levels and the roles of the metabolites should be informative. At present, there is little information on the lipooxygenase-dependent pathways and the role of docosahexaenoic acid.

One last possible explanation for the decreased plasma levels of polyunsaturated fatty acids in preeclampsia in this study may be altered storage and mobilization of these fatty acids, i.e., there may be increased incorporation of fatty acids into cell membrane phospholipids or a decreased release of fatty acids from lipid storage pools. Under normal conditions it is known that eicosapentaenoic acid and arachidonic acid are rapidly incorporated into platelet and endothelial cell membrane phospholipids.[11, 12] In disease conditions such as preeclampsia, a disequilibrium in the uptake and mobilization of such fatty acids from their storage pools may be an important pathophysiologic feature.

Arachidonic acid levels were not significantly different among any of the three groups studied and, since linoleic acid is low in the preeclamptic group, there could be increased metabolism of linoleic acid to arachidonic acid in that group. This is important since linoleic and arachidonic acids are the precursors to thromboxane and prostacyclin. Ogburn et al.[9] also reported no difference between the arachidonic acid levels of normal pregnant and preeclamptic patients. A decrease in the plasma arachidonic acid levels therefore do not appear to be responsible for manifestations of preeclampsia. Instead, these findings support other suggestions that the rate of production of a metabolite

may be more predictive of disease than the actual amount of the metabolite.[13] Recent studies have supported the use of aspirin to prevent preeclampsia by inhibiting cyclooxygenase conversion of arachidonic acid to thromboxane.[14] Decreasing thromboxane synthesis in this manner may counterbalance altered production of arachidonic acid in preeclamptic patients and restore the balance between prostacyclin and thromboxane.

The most significant difference in the *n*-3 class was noted in eicosapentaenoic acid, which is increased in the plasma of normal pregnant patients compared with that of nonpregnant patients but is significantly decreased in preeclamptic patients when compared with normal pregnant patients. Docosahexaenoic acid is also higher in normal pregnant patients. These two fatty acids play a significant role in regulating vascular activity via several mechanisms.[3] First, they can inhibit the synthesis of arachidonic acid from linoleic acid by competing for cyclooxygenase activity. Second, they can compete with arachidonic acid for incorporation in the 2-position in membrane phospholipids, reducing plasma and cellular levels of arachidonic acid.[12] Third, eicosapentaenoic acid competes with arachidonic acid as a substrate for cyclooxygenase activity and inhibits production of physiologically active thromboxane A_2 in platelets while producing only small amounts of thromboxane A_3, which is less of a platelet aggregator than thromboxane A_2.[15, 16] Thus in normal pregnant patients an increase in the levels of eicosapentaenoic acid and docosahexaenoic acid, as demonstrated in this study, may represent a normal physiologic response to pregnancy. In contrast, a reduced level of eicosapentaenoic acid in the preeclamptic women can cause altered vasodilatory control by significantly altering arachidonic acid synthesis and metabolism which, in turn, directly affects synthesis and metabolism of thromboxane. Eicosapentaenoic acid is also a precursor to prostanoids such as PGI_3, which are known vasodilators, and reduced levels of eicosapentaenoic acid could lead to more vasoconstriction and platelet aggregation.

Increasing the dietary intake of the *n*-6 and *n*-3 precursors during pregnancy, such as by taking fish oil supplements, may correct deficiencies or imbalances between different fatty acids and might ameliorate clinical manifestations of preeclampsia, just as it has in chronic essential hypertension. Our current results lend support to such therapy. Since it has been suggested that preeclampsia is an endothelial cell dysfunction with cell damage that can bring about a cascade of events resulting in coagulopathy and vasoconstriction,[17] fish oil supplements rich in *n*-3 fatty acids may be beneficial by decreasing platelet aggregation, reducing triglyceride levels to prevent triglyceride accumulation, which damages endothelial cells, and by increasing antithrombin levels. If preeclampsia is, in fact, a failure of trophoblast infiltration of the spiral arteries,[18] then dietary modifications begun in early pregnancy or before conception may modify the course of the disease. Although low-dose aspirin therapy has no known adverse fetal or adult effects,[19] therapy by dietary manipulation could be safer, particularly in the first trimester.

Additional studies are needed to define the roles of *n*-3 and *n*-6 polyunsaturated fatty acids in normal and preeclamptic pregnancies and, in particular, their effects on endothelial cell functions. At the present time, it is uncertain if the *n*-3 or the *n*-6 class of fatty acids is the more important in the pathophysiologic composition of preeclampsia. It is also uncertain if changes in the levels in either class are the cause of the disease, a sign of the disease, or a reflection of yet another metabolic imbalance.

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Amniotic fluid glucose and intraamniotic infection

Brian Kirshon, MD, Bernard Rosenfeld, MD, Giancarlo Mari, MD, and Michael Belfort, MD

Houston, Texas

Thirty-nine patients with either premature labor and/or preterm premature ruptured membranes underwent transabdominal amniocentesis to enable the following amniotic fluid analyses to be performed: culture and sensitivity, Gram's stain, and glucose determination. All nine patients with intraamniotic infection had amniotic fluid glucose values <10 mg/dl. Three patients with amniotic fluid glucose levels <10 mg/dl but without chorioamnionitis were delivered of infants within 72 hours of admission. The mean amniotic fluid glucose level of patients with intraamniotic infection (5 ± 2.4 mg/dl) was significantly lower than in those without intraamniotic infection (39.8 ± 18.42 mg/dl). All patients with amniotic fluid glucose values <10 mg/dl had either bacteria and/or white glood cells on Gram's stain. Two patients without chorioamnionitis had white cells on Gram's stain and amniotic fluid glucose values >10 mg/dl. It appears that amniotic fluid glucose is more sensitive and more specific than Gram's stain in the diagnosis of intraamniotic infection. All 12 patients with low amniotic fluid glucose values were delivered of infants within 72 hours as the result of either the presence of infection or the progression of labor. (Am J Obstet Gynecol 1991;164:818-20.)

Key words: Amniotic fluid, glucose, infection

Prematurity is the major cause of perinatal morbidity and mortality. Intrauterine infection is an important causative factor. However, a clear understanding of the role of infection is hampered by traditional tests, which are often insensitive and nonspecific.[1] Clinical signs and symptoms do not correlate well with the gold standard criteria of chorioamnionitis, which are considered to be either a positive amniotic fluid culture or polymorphonuclear leukocytes that extend through the fetal membranes and/or the umbilical cord.[2] Romero et al.[3] demonstrated that Gram's stain examination of amniotic fluid had a sensitivity of 79% and a specificity of 99.6% for the detection of microbial invasion of the amniotic cavity. The Gram's stain is a more rapid assessment for intraamniotic infection than is the conventional amniotic fluid culture. The purpose of this study was to assess the value of amniotic fluid glucose in the diagnosis of intraamniotic infection.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Baylor College of Medicine.
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Reprint requests: Brian Kirshon, MD, Baylor College of Medicine, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, One Baylor Plaza, Houston, TX 77030.
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Material and methods

Thirty-nine patients at <34 weeks of gestation with preterm labor and/or premature ruptured membranes were included in the study. Patients were excluded if they had diabetes or had received antibiotics in the past 7 days or if they were in advanced labor with cervical dilatation >4 cm. All patients underwent an

Table I. Glucose concentration

	Glucose concentration (mg/dl)	
Chorioamnionitis	*Amniotic fluid*	*Maternal serum*
Present	5 ± 2.4	89.6 ± 14.06
Absent	39.8 ± 18.42	98.3 ± 30.75
p Value	<0.005	NS

ultrasonography-guided amniocentesis with a 20-gauge spinal needle via an aseptic technique. The amniotic fluid was sent for glucose concentration, Gram's stain, culture, and sensitivity analyses, which included cultures for *Mycoplasma hominis* and *Ureaplasma urealyticum*. Glucose determination was performed by the glucose oxidase method, which is valid at low levels. A concomitant maternal serum glucose value was obtained. Intraamniotic infection was defined as a positive amniotic fluid culture and placental pathologic condition that revealed polymorphonuclear leukocytes that extended through the fetal membranes and/or the umbilical cord. A positive Gram's stain was defined as the presence of bacteria on Gram's stain. The amniotic fluid was not centrifuged. The correlation between intraamniotic infection and amniotic fluid glucose was analyzed. An unpaired Student *t* test was performed for statistical evaluation, and $p < 0.05$ was regarded as significant.

Results

The mean age, gravidity, and parity of patients with intraamniotic infection was 26.1 ± 5.2, 2.7 ± 1.4, and 0.6 ± 0.5, respectively. These values were not significantly different from those of patients without infection, namely, 24.8 ± 6.4, 2.2 ± 1.3, and 0.6 ± 0.9. The mean gestational age of all patients, which was similar in those with intraamniotic infection and in those without infection, was 31.9 ± 4.9 weeks. Nine patients met the criteria for intraamniotic infection, which included both a positive amniotic fluid culture and pathologic findings of the fetal membranes and umbilical cord. Thirty patients studied had no evidence of infection. Those patients with infection had a significantly lower amniotic fluid glucose level, whereas the maternal serum glucose value was not different between the two groups (see Table I). Twelve patients had amniotic fluid glucose values <10 mg/dl. All nine patients with infection had amniotic fluid glucose values <10 mg/dl. The three patients with amniotic fluid glucose values <10 mg/dl in the absence of infection were delivered of infants within 72 hours of admission. All patients with amniotic fluid glucose values <10 mg/dl had either bacteria or white blood cells on Gram's stain or both. Two patients without chorioamnionitis had white blood cells on Gram's stain, as well as amniotic fluid glucose levels >10 mg/dl. Tables II and III further outline the significance of amniotic fluid glucose in the presence of amniotic fluid infection. Six of the nine patients with intraamniotic infection had a positive Gram's stain.

Table II. Chorioamnionitis with amniotic fluid glucose level <10 mg/dl

Positive predictive value	9/9 (100%)
Negative predictive value	27/30 (90%)
Sensitivity	9/12 (75%)
Specificity	27/27 (100%)

Table III. Chorioamnionitis amniotic fluid glucose levels <10 and >10 mg/dl*

	Amniotic fluid glucose	
	<10 mg/dl	*>10 mg/dl*
Chorioamnionitis	9	0
No chorioamnionitis	3*	27

*All of these patients were delivered of infants within 72 hours, despite the absence of chorioamnionitis.

Comment

The incidence of positive amniotic fluid cultures of patients who are in preterm labor varies from 0 to >33%.[4-13] Unfortunately, many of these studies have small numbers of participants. However, positive amniotic fluid culture with chorioamnionitis is one undisputed cause of preterm labor and delivery. A poor correlation between amniotic fluid Gram's stain and culture as found in the current study has been previously reported.[14] False-negative Gram's stains occur with a low organism inoculum ($<10^5$ colony-forming units/ml).[4] Romero et al.[4] failed to increase the sensitivity of the Gram's stain by concentrating the number of organisms with ultracentrifugation of the specimen before the Gram's stain was done. A false-positive Gram's stain may occur when fastidious organisms are seen on the Gram's stain and fail to grow on culturing. Skoll et al.[14] did find a correlation between preterm delivery and a false-negative Gram's stain.

The leukocyte esterase test has been reported to be valuable in the diagnosis of bacterial colonization of amniotic fluid.[15] This rapid, inexpensive test detects neutrophil activity in urine and in other fluids.[16] Hoskins et al.[16a] reported a 91% sensitivity and a 95% specificity of the leukocyte esterase test in the diagnosis of chorioamnionitis. The presence of white blood cells per se in amniotic fluid may[4] or may not[14] be associated with intraamniotic infection. It may be that white blood cell activity as demonstrated by the destructive leukocyte esterase enzyme is the important factor in deter-

mining the presence of intraamniotic infection. Also, the presence of a low intraamniotic fluid glucose level appears to correlate with intraamniotic infection.

Histologic chorioamnionitis has been reported to be associated with amniotrophism that is displayed by polymorphonuclear leukocytes in the extraplacental membranes, placenta, and umbilical cord.[17] The premise on which this is based suggests that diffusible leukotaxic factors are released into the amniotic fluid as part of the inflammatory response.[18] Pankuch et al.[17] found a positive leukotaxic response to be a better predictor of histologic chorioamnionitis than either a positive Gram's stain or an amniotic fluid culture. It is possible that the consumption of amniotic fluid glucose by the leukocytes in response to the amniotrophism could account for the low amniotic fluid glucose levels in this study. Additional study in this regard is required.

This preliminary study indicates that amniotic fluid glucose analysis may be more sensitive and specific than Gram's stain in the diagnosis of intraamniotic infection. The amniotic fluid glucose level was significantly lower in patients with evidence of intraamniotic infection than in patients without infection. Measurement of amniotic fluid glucose is an inexpensive, rapid test that can be performed on amniotic fluid to determine the presence of intraamniotic infection. This test may be considered a helpful addition to Gram's stain, the leukocyte esterase test, and amniotic fluid culture in the identification of intraamniotic infection.

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Fetal breathing movements after preterm premature rupture of membranes

Alistair B. Roberts, MD, Israel Goldstein, MD, Roberto Romero, MD, and John C. Hobbins, MD

New Haven, Connecticut

Forty-three patients with preterm premature rupture of membranes were studied between 24 and 34 weeks' gestation. Intraamniotic infection was excluded by amniocentesis. There were significantly fewer fetal breathing movements for the first 2 weeks of membrane rupture, compared with controls, with a return to near normal by the third week. This reduction in fetal breathing may play a role in the production of pulmonary hypoplasia in some of these patients. Further studies are needed to investigate whether ruptured membranes are associated with a reduction in fetal breathing at earlier gestational ages. (Am J Obstet Gynecol 1991;164:821-5.)

Key words: Premature rupture of membranes, ultrasound, fetal breathing, pulmonary hypoplasia

Preterm premature rupture of membranes before 24 weeks' gestation is associated with a high perinatal mortality rate.[1] The major causes of death are complications of prematurity, infection, and pulmonary hypoplasia.[2-4] The cause of pulmonary hypoplasia in this setting is still debated, with the two main theories being loss of fetal lung fluid[5] and inhibition of normal fetal breathing.[6]

Fetal breathing movements are considered important for normal lung development. Indeed denervation of the fetal diaphragm in the fetal lamb eliminates fetal breathing movements and impairs lung growth and development.[7, 8] There is conflicting evidence on the role of fetal breathing in the development of pulmonary hypoplasia in human fetuses with preterm premature rupture of membranes. Blott et al.[9] have reported absence of fetal breathing in fetuses that subsequently die of pulmonary hypoplasia. In contrast, Moessinger et al.[10] found no difference in the time spent breathing between fetuses with or without pulmonary hypoplasia.

We have initiated a series of studies to formally address the role of fetal breathing movements in the development of pulmonary hypoplasia. As a first step, we decided to determine the effect of ruptured membranes per se on fetal breathing. Surprisingly there is a paucity of data regarding this important issue. Previous studies in this area have not been able to control for some of the factors known to affect fetal breathing, of which intraamniotic infection is one of the most important.[11] Since amniocentesis is routinely used in this institution in the management of patients with premature rupture of membranes, this afforded the unique opportunity to study fetal breathing in a population where subclinical intraamniotic infection had been reliably excluded.

From the Department of Obstetrics and Gynecology, School of Medicine, Yale University.
Received for publication July 12, 1990; revised October 1, 1990; accepted October 15, 1990.
Reprint requests: Alistair Roberts, Department of Obstetrics and Gynecology, University of Auckland, National Women's Hospital, Claude Road, Auckland 3, New Zealand.
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Material and methods

Patient population. Patients were selected from a data base of 90 patients with preterm premature rupture of membranes occurring before 34 weeks' gestation. A previous publication from this department was based on some cases in this file.[11] It addressed the usefulness of the study of fetal activity in detecting intraamniotic infection. The objective of the study was to determine the effect of ruptured membranes per se on fetal breathing. We therefore excluded patients with conditions known to affect fetal breathing.[12]

Inclusion criteria. Patients were included if they met the following criteria: (1) gestational age of 24 to 34 weeks with a singleton pregnancy; (2) no maternal disease or drug usage; (3) clinical and laboratory documentation of premature rupture of membranes (ferning, Nitrazine test, and pooling of fluid); (4) no contractions, evidence of labor, or delivery within 24 hours of the examination; (5) amniocentesis to exclude intraamniotic microbial invasion of the amniotic cavity; and (6) a neonatal birth weight above the tenth percentile for gestational age.[13]

Amniotic fluid microbiologic composition. Amniotic fluid was cultured for aerobic and anaerobic bacteria, *Mycoplasma hominis*, and *Ureaplasma urealyticum* by

Table I. Fetal breathing and trunk movements in patients with 1 week of ruptured membranes compared with normal controls

	A. PROM	*B. PROM*	*C. Control*	*D. PROM*	*E. Control*
Gestation (wk)	24-28	28-31	28-31	31-34	31-34
No. of patients (%)	9	15	15	18	16
Breathing					
Mean	2.6	9.1	26.5	13.3	39.2
Median	0.7*	1.2†	26.0	10.7†	39.5
SD	4.4	14.3	25.6	11.2	24.4
Movements (%)					
Mean	19.4	15.9	19.0	10.4	17.0
Median	12.2	14.2	17.2	7.0†	15.5
SD	15.5	11.2	9.0	6.6	5.0
Days of ruptured membranes (mean)	3.0	2.0	—	1.9	—

PROM, Premature rupture of membranes.
*No controls but normal for 24 to 28 weeks according to literature (8.5%).[16]
†Significantly different from controls.

methods previously described.[11] Patients with positive cultures for any of these microorganisms were excluded from this study. Amniocentesis was standard clinical management in the department. An amniocentesis was performed within 24 hours of admission, if possible, or after bed rest to try to prevent amniotic fluid drainage so as to permit amniocentesis. The amniocentesis was repeated weekly or more frequently if infection was clinically suspected.

Fetal activity recordings. Fetal breathing and fetal trunk movements were studied with real-time ultrasonography. Studies were performed 30 to 120 minutes after breakfast. All patients were on a normal diet. Ultrasonographic recordings were of 30 minutes' duration. These were videotaped, and the amount of breathing and trunk movements was measured with a stopwatch. All breathing movements were recorded, except for isolated single breaths. The beginning and end of each breathing session were timed with a stopwatch, and the total amount of time the fetus spent breathing in each session was expressed as a percentage. Trunk movement was recorded in the same manner with a stopwatch used to record the beginning and end of each trunk movement. Tapes were analyzed the same day with no knowledge of the amniocentesis results.

Recordings used in this study were those made within 24 hours of an amniocentesis; a total of 64 recordings were obtained in 43 patients. Seventy-one percent of the recordings were made before amniocentesis.

Study design. To determine the effect of ruptured membranes on fetal activity, it was necessary to allow for changes caused by gestational age. We therefore divided the patients into three groups: (1) 24 to 27 weeks, (2) 28 to 31 weeks, (3) 32 to 34 weeks. Normal fetuses from women with matched gestational ages and intact membranes were used as controls.[14] Only one recording per patient (that obtained during the first week) was selected for each gestational age interval.

Two approaches were used to describe changes in fetal activity over time: a longitudinal and a cross-sectional approach. Eight patients had serial recordings over 3 weeks within the interval of 28 to 34 weeks. They provided the data for longitudinal observations.

For the cross-sectional study patients were divided into three groups according to the duration of ruptured of membranes (1 week, 2 weeks, and 3 weeks). One recording per patient per week interval was used. The control group consisted of normal women with intact membranes between 28 and 34 weeks' gestation.

Statistical analysis. For all cross-sectional comparisons only one recording per patient was used. Comparisons were made between the study group (rupture of membranes) and the control group (intact membranes). A Wilcoxon rank sum test was used to analyze fetal activity as the data were not normally distributed. Longitudinal observations were analyzed with a randomization test for matched samples.[15] Calculations were performed with the True Epistat program (Epistat Services, Richardson, Tex.).

Results

Fetuses with ruptured membranes spent significantly less time breathing than those with intact membranes (mean, 14.3%; median, 9.4%; range, 0 to 93% vs mean, 33%; median, 27%; range, 0 to 90%; respectively; $p < 0.001$). The amount of time spent breathing was significantly reduced at all gestational ages from 24 to 34 weeks as illustrated in Table I.

The results of the longitudinal study are displayed in Fig. 1 and Table II. The amount of time spent breathing increased with the duration of rupture of membranes ($p = 0.06$). In contrast, no change occurred in trunk movement over time. These observations were confirmed by cross-sectional analysis (Table III). The amount of time spent breathing was significantly less in the first 2 weeks of membrane rupture

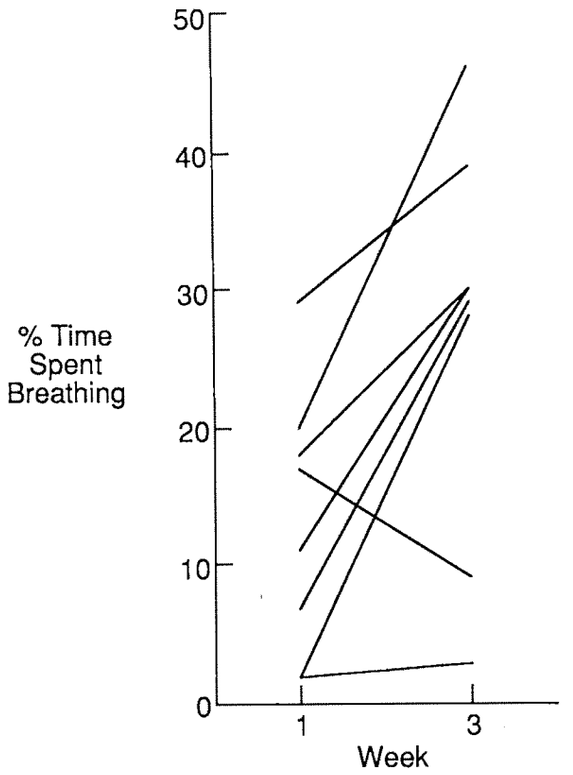


Fig. 1. Percentage of time spent breathing at 1 week and 3 weeks of membrane rupture in eight fetuses in longitudinal study.

compared with the control group ($p < 0.001$ and $p < 0.01$, respectively). In contrast, no difference was observed in time spent breathing between the group in the third week of membrane rupture and the control group. Of considerable interest is that the total duration of fetal trunk movement was not different from the control at any interval.

No significant difference was found between the amounts of fetal breathing or fetal movement in recordings made before and after amniocentesis.

Comment

Our data clearly indicate that rupture of membranes was associated with a significant reduction in the amount of time the fetus spent breathing. This effect was dramatic and occurred at all gestational ages examined from 24 to 34 weeks' gestation. The reduction in breathing lasted for at least 2 weeks and then returned to normal values. In the majority of cases this was not accompanied by a similar reduction in fetal trunk movements.

Why should rupture of membranes be associated with a significant reduction in fetal breathing activity? Since the phenomenon is not associated with decreased fetal trunk activity, rupture of membranes does not seem to produce a generalized depression in fetal motor activity. Several mechanisms could be invoked to explain our findings, including infection, labor, and hypoxia. Subclinical microbial invasion of the amniotic cavity has been associated with a significant reduction in fetal breathing activity in the setting of preterm premature rupture of membranes.[11] This possibility has been excluded as all patients had a negative amniotic fluid culture for bacteria and mycoplasma species around the time of study. None of our patients were in labor or were delivered within 24 hours of the study. Hypoxia was unlikely to play a major role, because these fetuses did not have any evidence of fetal distress, fetal trunk movements were not affected, and there was no evidence of growth retardation. Amniocentesis has been reported to reduce fetal breathing activity for 48 hours.[17] No evidence could be found in this study that amniocentesis in patients with premature rupture of

Table II. Matched pairs: Recordings in first and third weeks after ruptured membranes

	Week 1	*Week 3*
No. of patients (%)	8	8
Breathing (%)		
Mean	13.1	27.6
Median	13.9	24.9*
SD	9.7	20.5
Movements (%)		
Mean	23.7	18.7
Median	17.9	18.0
SD	17.0	6.8
Gestation (wk)	29.3	31.5

*One-tailed, $p = 0.03$; two-tailed $p = 0.06$.

Table III. Breathing movements and trunk movements in premature rupture of membranes with patients between 28 and 34 weeks' gestation, grouped by number of weeks that membranes were ruptured

	Week 1	*Week 2*	*Week 3*	*Controls*
No. of patients	33	11	11	31
Breathing (%)				
Mean	11.4	17.0	25.1	33.0
Median	7.1*	10.0*	21.0	27.0
SD	12.6	26.2	17.4	25.4
Movements (%)				
Mean	13.4	19.6	18.7	18.0
Median	12.0	14.2	18.0	16.5
SD	9.9	17.6	6.8	7.0
Gestation (wk, mean)	31.4	31.3	31.5	31.0
Days ruptured (mean)	2.6	8.3	17.3	—

*Significantly different from controls.

membranes further reduced fetal breathing activity. In the majority of recordings the amniocentesis was performed after the recording of fetal activity.

These findings have clinical and physiologic implications. Fetal breathing and fetal movements are part of the biophysical profile that has been advocated for the diagnosis of infection in patients with preterm premature rupture of membranes. Whereas the presence of fetal breathing has a high negative predictive value for intraamniotic infection, a depression in fetal breathing activity is nonspecific. Our data indicate that preterm premature rupture of membranes in the absence of infection is associated with a significant and prolonged reduction in fetal breathing. Fetal trunk movement is significantly reduced in the presence of intraamniotic infection but not in rupture of membranes per se and therefore may be more useful in diagnosing infection in fetuses with depressed fetal breathing activity. The vast majority of fetuses with intraamniotic infection will have reduction in both breathing and trunk movements, and we have shown that the measurement of such parameters is useful clinically.[11]

The physiologic implications of a 2-week reduction in fetal breathing revolve around the subject of lung growth. There are conflicting reports about the role of fetal breathing in pulmonary hypoplasia. Studies by Blott et al.[9, 18] noted that fetuses with premature rupture of membranes and pulmonary hypoplasia had reduced amounts of fetal breathing. However, interpretation of these findings is difficult because of the definition of "breathing" used in the study and because the mean gestational age at delivery was 26 weeks. Breathing was considered to be present if thoracic movements were present for a continuous period of 60 seconds in a recording session of 30 to 120 minutes. We are unaware of data indicating what proportion of normal fetuses breathe for 60 seconds continuously before 24 weeks' gestation. One study has indicated that only 59% of normal fetuses have 60 seconds of continuous breathing in a 30-minute recording between 25 and 28 weeks' gestation.[19] In contrast, Moessinger et al.[10] studied 10 patients with prolonged ruptured membranes from 25 to 35 weeks' gestation and found no difference in time spent breathing between fetuses with and without pulmonary hypoplasia. This study used a 60-minute recording period and measured the percentage of time spent breathing. Breathing was defined as starting when there was a breath followed by two others or more within a 6-second window. Interestingly, in the cases without pulmonary hypoplasia the percentage of time spent breathing was significantly less than that of controls between 30 and 35 weeks' gestation.

Animal studies have suggested that both loss of lung fluid and inhibition of fetal breathing can lead to pulmonary hypoplasia and that the effects are additive.[5] Studies in the guinea pig also suggest that even short periods of oligohydramnios can interfere with lung growth.[20] It is possible therefore that a 2-week reduction of fetal breathing in the human fetus at a critical period in lung growth and development might lead to pulmonary hypoplasia. Thibeault et al.[21] concluded that pulmonary hypoplasia in the human fetus could develop after only 6 days of membrane rupture.

This study investigated the effect of prolonged rupture of the membranes on fetal breathing at 24 to 34 weeks' gestation. However, the critical period in human fetal lung growth is before 24 weeks' gestation. Further studies are needed to investigate whether there is inhibition of breathing at earlier stages of gestation. There is also a need for more information on normal patterns of fetal breathing before 24 weeks.

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Outpatient sedation: An essential addition to gynecologic care for persons with mental retardation

David A. Rosen, MD,[a] Kathleen R. Rosen, MD,[a] Thomas E. Elkins, MD,[b] H. Frank Andersen, MD,[b] S. Gene McNeeley, Jr., MD,[b] and Cheryl Sorg, RN[b]
Ann Arbor, Michigan

Routine gynecologic care for persons with mental retardation may be difficult to provide, especially to those women who do not allow a pelvic examination to be performed. Of 275 women referred to a multidisciplinary clinic addressing the reproductive health concerns of mentally retarded women, 61 patients (22%) did not allow a gynecologic examination to be performed. The administration of ketamine alone, midazolam alone, or a combination of midazolam and ketamine allowed for the successful performance of a gynecologic examination in 81% of previously uncooperative women. No adverse effects of the medications were noted. We conclude that sedation of difficult-to-examine, mentally handicapped women can be safely performed in the outpatient setting, thus avoiding the need for general anesthesia and its inherent risks. (Am J Obstet Gynecol 1991;164:825-8.)

Key words: Mental handicap, sedation, ketamine, midazolam, oral sedation

Routine outpatient gynecologic care for persons with mental retardation, including a pelvic examination, is legally mandated by most states but has been difficult to provide in many settings and has been relatively unobtainable for many patients. The lack of cooperation during attempted pelvic examinations, concerns about the use of physical force or excessive coercion, and the incompleteness of pelvic examinations in uncooperative patients have been identified by primary care providers as the leading reasons for the avoidance of the outpatient evaluation of women with mental retardation.[1] This reluctance by the primary care provider to perform pelvic examinations in this population has resulted in >40% of initial visits to the gynecology clinic at the University of Michigan for persons with mental retardation being scheduled as the person's first pelvic examination.[2] In an effort to enhance the availability of routine gynecologic care for persons with mental retardation, a program for outpatient sedation was begun. The sedation program briefly mentioned in prior publications is fully described in this report.[3]

Only a few isolated case reports exist concerning the sedation of persons with mental retardation in any age group.[4,5] Guidelines have been established for the practice of sedation in the medical specialties of pediatrics, dentistry, and radiology.[6,7] However, these documents do not evaluate the safety or efficacy of individual sedative regimens.

The purpose of this study was to evaluate the safety and efficacy of oral ketamine and/or midazolam for the sedation of the difficult-to-manage patient with mental retardation in an outpatient gynecologic setting.

From the Department of Anesthesia, Pediatrics and Communicable Disease[a] and the Department of Obstetrics and Gynecology,[b] University of Michigan Medical Center.
Received for publication April 12, 1990; revised August 23, 1990; accepted October 25, 1990.
Reprint requests: Thomas E. Elkins, MD, University of Michigan Medical Center, 1500 E. Medical Center Dr., Department of Obstetrics and Gynecology, D2234 MPB, Box 0718, Ann Arbor, MI 48109-0718.
6/1/26708

Table I. Parameters of sedation scale

Sedation scale					
1	*2*	*3*	*4*	*5*	*6*
Normal	Calm but with full muscle tone	Drowsy with mild muscle relaxation	Drowsy with physically relaxed muscle tone	Eyes closed; responds to minor stimulation; airway reflexes intact	Asleep; does not respond to stimulation; airway reflexes absent

Methods

This study received institutional approval by the human use and ethics committees. Mentally handicapped women in whom previous attempts at performing a gynecologic examination had been unsuccessful were entered into this study as an alternative to examination while they were under general anesthesia. Examination and the sedation were performed in the outpatient gynecology clinic. Patients received nothing by mouth for 6 hours before the clinic appointment. (This is easily monitored in the highly supervised and activity-structured living environments of the patients in this study.) An anesthesiologist was present to administer the medications and monitor the patients who remained under continuous medical observation during the study period. Heart rate, blood pressure, respiratory rate, and level of sedation were measured every 15 minutes. Sedation was evaluated before the examination and measured with a six-point scale (see Table I). No change in mental status as compared with baseline was recorded as a score of 1. A patient with eyes closed received a score of 5, whereas a deeply sedated patient would receive a score of 6. Oxygen, suction, and airway and resuscitation equipment was available. A primary directive of the clinic was to be as nonthreatening as possible; therefore no sedative medications were given by parenteral routes.

The initial dose of all medications was administered by the oral route. Subsequent medications were given intranasally if this route was acceptable to the patient or orally if the patient refused intranasal medication. Three regimens were studied: ketamine alone, 4 to 10 mg/kg; midazolam alone, 0.1 to 0.4 mg/kg; and ketamine, 4 mg/kg plus midazolam 0.4 mg/kg. Unflavored liquid preparations of these drugs were mixed with either flavor extracts or chocolate syrup to facilitate acceptance of oral medication. Intranasal medications were not diluted. Adequacy of sedation was assessed every 15 minutes. Gynecologic examination was attempted when a sedation score ≥ 3 was achieved. If the sedation score was still <3 at 45 minutes, an additional sedative dose was given. The occurrence of any adverse side effects from the sedatives was noted. Patients were discharged from the clinic when they had resumed their usual level of functioning.

The following additional patient demographic data were recorded: patient's age and weight, medication and allergy history, prior visits to the sedation clinic, time until return to normal function, and level of cognitive function. The patient's functional status was gauged on a five-point scale. A score of 1 indicated minimal impairment, whereas a score of 5 indicated a severe handicapping condition (see Table II).

The majority of the patients (60%) were very severely impaired (score = 5). Twenty percent of the women were less severely handicapped (score = 4), whereas patients with moderate handicap (score = 3) made up the remaining 20% of the population.

Results

During the 18-month study period, 275 patients were referred to the model clinic for gynecologic consultation. Sixty-one patients (22%) were scheduled for a total of 102 examinations with the sedation protocol, and 52 of these 61 patients (81%) were successfully examined. A total of 85 of these 102 visits resulted in successful examinations.

Gynecologic examination was possible after only one dose in 47 patient visits, 25 patients requiring a second dose were successfully examined, and only 13 patients required a third sedative dose before examination was possible. The initial regimen selected was ketamine in 60%, midazolam in 34%, and a combination in 6%. There was no significant difference in the occurrence of adequate sedation as a function of the initial medication selection. If a second dose was required, midazolam alone was chosen in two thirds of cases regardless of the initial medication. All patients with moderate mental handicap were successfully examined. The mean ketamine dose was 8.0 mg/kg. The mean dose for those patients who received midazolam as the primary agent was 0.46 mg/kg, whereas those who received the combination of midozalam and ketamine had a mean dose of 0.34 mg/kg. Although the means differed slightly for those who received midazolam, this difference was not statistically significant.

A successful gynecologic examination was considered to be one in which a bimanual examination was allowed for the adequate palpation of pelvic organs or one that excluded significant pelvic abnormalities. A speculum

Table II. Parameters of mental function scale

Mental function scale				
1	*2*	*3*	*4*	*5*
Upper-moderate motor and/or mental disability	Moderately mentally handicapped	Severely mentally handicapped	Severely mentally handicapped with some physical disability	Profoundly mentally handicapped with some associated physical disability

examination was successfully performed in only 52%. However, Papanicolaou smears were obtained from 87% of women during speculum examinations or by guiding the cotton swab into the cervical os with one finger in the vagina. Examinations were initially attempted when the patient reached a level of 3 on the sedation score. The maximum sedation score received by any of the patients was 5. This occurred in 16% of patient visits.

All patients with moderate mental handicap were examined. In patients with more than moderate mental handicap, failure to complete an examination occurred for two reasons: (1) One patient was well sedated, but increased muscle tone impeded the examination during three consecutive clinic visits. This patient returned to the sedation clinic on one occasion after the examination while she was under general anesthesia and refused all sedation medication. (2) Some patients refused a repeat medication dose. Eight patients refused supplemental oral or nasal medication during 13 clinic visits.

In the nine patients who could not be examined, the failure to perform an examination resulted in a total of eight examinations with patients under general anesthesia. Three of these examinations were in the same patient, who was undergoing follow-up of an underlying gynecologic condition. In three of the patients with "failure," the need for more complete examinations at the time was outweighed by the risks of general anesthesia.

A significant change in blood pressure, heart rate, or respiratory rate was not demonstrated in any patients. None of the patients received any parenteral sedative medications. Patients had no difficulties in supporting their own airways, and there was no need to secure intravenous access in any patient. Conjunctival erythema was observed when sedation was achieved and dissipated as the patient returned to normal functioning. Nausea with or without vomiting was the most frequent side effect observed. The incidence of nausea decreased from 38% to 21% when the oral medications were mixed in chocolate syrup rather than flavor extract. Of those who did vomit, only 35% had more than one episode. Vomiting usually occurred before examination (before a sedation level of 3 was obtained). No patient manifested any agitation or signs of dysphoria or experienced an increase in oropharyngeal secretions. No airway obstruction occurred. Prolonged lethargy (sedation score ≥3) for 12 hours after the procedure was noted on three occasions. The median time from examination to discharge from the clinic was 45 minutes. Eighty percent of patients were discharged after 1 hour, 16% after 90 minutes, and the remainder by 2 hours.

Comment

This manuscript reports the development and evaluation of a sedative regimen for use in the mentally handicapped patient undergoing a gynecologic examination. The goal of this regimen was to provide safe, reliable, short-term sedation for use in an outpatient setting without using a parental route of administration. There is limited information available about either the nasal or oral route of administration for ketamine and/or midazolam in the normal adult population.[8, 9] However, these agents are routinely administered by these routes as preoperative sedatives in pediatric and adolescent patients, where excellent results have been achieved.[10] Our study demonstrates that similar results can be achieved with orally and nasally administered ketamine and midazolam in patients with mental retardation.

Grant et al.[8] describe the pharmacokinetics after a single oral administration of ketamine 0.5 mg/kg. Peak plasma concentrations occurred at 45 minutes and none of the patients lost consciousness. Ketamine was chosen because of its short duration of action and its capacity to maintain hemodynamic stability. In non–mentally handicapped patients, low-dose ketamine (0.5 mg/kg) allows maintenance of airway reflexes and also produces minimal respiratory depression. This study has demonstrated that in patients with mental retardation an elevated dose (8 mg/kg) has proved effective and offers the same advantages mentioned previously.

Dysphoric reactions have been noted when the drug is given parenterally to adults, but adverse effects after an oral sedative dose of ketamine have not been reported in normal adults.[8] Hollister and Burn[11] noted that the incidence of unpleasant dreams and hallucinations is higher in older children and adults than in

younger ones. It may be that this dysphoric reaction requires higher levels of cognition. This may explain why children and perhaps some persons with mental retardation have a lower incidence of dysphoric reaction to ketamine than do adults. The mentally retarded patients in this study required a mean total oral ketamine dose of 8 mg/kg to produce effective sedation, and peak effect was noted to occur at 45 minutes after administration.

Midazolam was chosen because it is a rapid-acting agent with a short duration of action. The oral administration of midazolam to adults has been investigated by Heizmann et al.[9] Absorption appears to occur rapidly with peak plasma levels reached within 30 minutes; however, bioavailability is quite variable (from 31% to 72%) because of the high liver extraction.[9] It has been effectively used via nonparenteral routes for preoperative sedation in children.[10]

At this time the major drawback of this regimen is that oral preparations for ketamine or midazolam are currently unavailable in this country. The parenteral solutions are quite bitter and unpalatable. In this study the vehicle (a flavored [cherry, grape, etc.] alcohol-containing extract) that was initially used to deliver the drugs produced an undesirably high incidence of nausea and/or vomiting. The occurrence of nausea decreased from 38% to 21% when the drugs were delivered in chocolate syrup; however, the proportion of patients who refused medication remained quite high (18%).

Ketamine and midazolam both have been used effectively for sedation by both oral and intranasal routes. The advantage of the intranasal route was a more rapid onset of effect (5 minutes for nasal, 30 minutes for oral). Only three of the patients would accept the nasal medications before receiving the oral medication. The rest required some sedative effect from the oral medication before they would allow nasal administration. Although there was interaction between midazolam and ketamine as demonstrated by the results, their effects did not appear to be simply additive. Ketamine appeared to be more effective in the severely handicapped patient, whereas midazolam was easier to titrate to appropriate levels of sedation in the less handicapped individual. As it may be difficult to predict when a patient will allow a pelvic examination, examination should be attempted when a level of 3 is reached. This will avoid the need for additional medication doses.

We were unable to successfully examine 9 of 61 patients. One of these patients could not be examined during four consecutive appointments. Three patients refused to accept the medication during one or more clinic visits. Five of the patients who would not tolerate the initial gynecologic examination were successfully sedated and examined during a subsequent clinic visit. Despite these sedation failures, the program is considered to be a success for all involved.

A sedation program such as we have described offers significant cost savings to the public. The average total hospital and physician charge for an examination under anesthesia is approximately $2000 at the University of Michigan Medical Center; in this series the approximate cost of each outpatient gynecologic examination that was performed with oral-nasal sedation was $60 to $80. Thus savings for the public were more than $100,000 and these handicapped women avoided the risks associated with general anesthesia.[12]

In summary, the above-described sedation program has greatly enhanced the ability of the gynecology service to provide humane, cost-effective care to previously unmanageable patients. However, we caution against the indiscriminate administration of these sedative agents.

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Does unexplained second-trimester (15 to 20 weeks' gestation) maternal serum α-fetoprotein elevation presage adverse perinatal outcome?

Pitfalls and preliminary studies with late second- and third-trimester maternal serum α-fetoprotein

Joe Leigh Simpson, MD, Sherman Elias, MD, Cynthia D. Morgan, RN, MS, Richard N. Andersen, PhD, Lee P. Shulman, MD, Baha M. Sibai, MD, and Amanda Skoll, MD

Memphis, Tennessee

Several reports have suggested that persons with an unexplained maternal serum α-fetoprotein elevation at 15 to 20 weeks' gestation are at an increased risk for a variety of other pregnancy complications (e.g., preeclampsia) and adverse perinatal outcomes (e.g., fetal death, low-birth-weight infants). However, ascertainment biases could explain some of these reported findings, and predictive value of unexplained elevated maternal serum α-fetoprotein levels in the prediction of pregnancy complications seems limited. If elevated second-trimester levels were truly predictive of pregnancy complications, we reason that third-trimester levels could prove even more useful. We thus studied late second-trimester and early third-trimester (24 to 36 weeks' gestation) maternal serum α-fetoprotein levels with the same enzyme immunoassay we use to evaluate routine second-trimester (15 to 20 weeks' gestation) levels. Values rose up to 32 weeks and fell slightly thereafter. Variance was greater than at 15 to 20 weeks but not so great as to preclude clinical usefulness in the third trimester. Of 279 women with a normal (0.4 to 2.49 multiples of the median) maternal serum α-fetoprotein value at 15 to 20 weeks' gestation, 270 (96.8%) showed levels in the same range later in gestation; however, none of six singleton pregnancies with unexplained maternal serum α-fetoprotein levels > 2.50 multiples of the median at 15 to 20 weeks' gestation showed maternal serum α-fetoprotein levels in this range at 24 to 36 weeks' gestation. The relationship between second- and third-trimester maternal serum α-fetoprotein levels in abnormal pregnancies remains to be elucidated in a large sample. Thus we are conducting not only cohort but also cross-sectional studies. Preliminary findings suggest that women with preterm premature rupture of membranes or with premature labor show elevated late second-trimester and early third-trimester maternal serum α-fetoprotein levels; however, larger sample sizes are necessary. (Am J Obstet Gynecol 1991;164:829-36.)

Key words: Third trimester, maternal serum α-fetoprotein, pregnancy complications, adverse outcome

Second-trimester maternal serum α-fetoprotein (AFP) screening for the detection of neural tube defects is accepted obstetric practice. However, relatively few maternal serum AFP elevations are actually associated with fetal neural tube defects. Erroneous gestational age assignment, multiple gestation, and fetal death are common explanations, and a host of rare explanations (e.g., congenital nephrosis) have been reported. However, there remain many pregnancies in which there is no ostensible explanation for elevated maternal serum AFP levels. The significance of unexplained elevations is uncertain, but several reports suggest that such persons are at an increased risk not only of pregnancy loss but of a variety of other pregnancy complications (e.g., preeclampsia) and adverse perinatal outcomes (e.g., low-birth-weight infants).[1-7] In fact, a committee of the American Society of Human Genetics stated that "women who have increased concentrations of MSAFP and whose fetus does not have a demonstrable abnormality by either level II ultrasound or karyotyping from amniotic fluid continue to have an increased risk for

From the Department of Obstetrics and Gynecology, University of Tennessee, Memphis.
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Reprint requests. Joe Leigh Simpson, MD, Department of Obstetrics and Gynecology, University of Tennessee, 853 Jefferson Ave., Memphis, TN 38163.
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having a fetus with major congenital malformation and poor pregnancy outcomes, such as perinatal death and low birthweight."[8]

Although not unaware of the potential utility of maternal serum AFP in the identification of women at increased risk of pregnancy complications, we are concerned that incorporation of the above recommendation into obstetric practice is premature.[9] Not only could ascertainment biases be responsible for some of the reported findings (see Comment), but sensitivity and specificity for use of unexplained elevated maternal serum AFP levels as a predictor of pregnancy complications are limited. Moreover, it remains to be determined whether those women with unexplained maternal serum AFP elevations who later manifest pregnancy complications are distinct from those already known clinically to be at increased risk on the basis of history (e.g., hypertension, vaginal bleeding, fetal growth discordant for dates). Such a characteristic would obviously need to apply before recommending routine (15 to 20 weeks' gestation) second-trimester maternal serum AFP examination for identification of pregnancy complications, yet this point appears not to have been raised in previous publications.

How can the obstetric significance of unexplained second-trimester maternal serum AFP best be determined? If elevated second-trimester maternal serum AFP levels were truly predictive of pregnancy complications, we reason that third-trimester maternal serum AFP levels could prove even more useful. All antepartum tests have a finite time for the prediction of poor outcome, and the maternal serum AFP test should be no exception. If a given test has value in the identification of pregnancy complications or adverse perinatal outcome, the predictive value should be higher near the time such complications are usually manifested. In fact, failure to find a relationship between elevated third-trimester maternal serum AFP and adverse perinatal outcome might cast doubt on claims of a relationship between unexplained elevated second-trimester maternal serum AFP levels and subsequent adverse perinatal outcome. In this article we shall first discuss the significance of unexplained elevated levels in the second trimester (15 to 20 weeks' gestation) and then present our preliminary findings concerning late second-trimester and early third-trimester (24 to 36 weeks' gestation) maternal serum AFP levels.

Methods and preliminary results

Assay for late second-trimester and early third-trimester maternal serum AFP levels. In our laboratory, the Abbott enzyme immunoassay is used for routine measurement of second-trimester maternal serum AFP. The average interassay coefficient of variation at 15.9 ng/ml is 6.5%; at 83.7 ng/ml, 7.3%; and at 219 ng/ml, 8.1%. This assay was used without modifications for this study of late second-trimester and early third-trimester maternal serum AFP.

Blood samples were obtained from unselected women receiving obstetric care at the Regional Medical Center (Thomas F. Gailor Clinics). In this large urban hospital (7800 deliveries per year), 80% of patients are black; 20% are white. Their socioeconomic status is predominantly low income to lower middle class. The proportion with obstetric complications is relatively high (e.g., 11% preeclampsia). Samples in this study were stored at −70° C until collection was completed and assays were performed in batches.

The interval of 24 to 36 weeks' gestation was chosen for the generation of late second-trimester and early third-trimester standards. Obstetric interventions would probably need to occur during this interval to affect pregnancy outcome favorably. Fig. 1, *A* and *B*, shows maternal serum AFP values that are not weight adjusted. Although weight adjustment is routine in second trimester, it is not clear whether adjustments used at 15 to 20 weeks' gestation would be appropriate later in gestation (24 to 36 weeks). Applying second-trimester weight adjustments did not substantively alter results; however, lack of certainty concerning validity of an extrapolated weight adjustment leads us to display raw data (Fig. 1). After pregnancies were completed, medical records were reviewed to identify subjects whose gestational age had been assigned incorrectly when the blood sample was obtained. In 43 subjects original dating appeared erroneous because the dating differed by >2 weeks from that based on the agreement of ultrasonographic examination later in pregnancy, delivery date, and birth weight. That is, the latter three were in agreement and contradictory to our original assignment. Fig. 1, *B*, shows maternal serum AFP values by gestational week before and after adjustment. No significant change in medians occurred as a result of this reassignment. That so few gestational ages were in error probably reflects our relatively frequent use of ultrasonography early in pregnancy. Nonetheless, in this article we use values based on uncorrected gestational age because such an approach is conventionally used to generate maternal serum AFP medians.

Fig. 1 demonstrates that maternal serum AFP level rises between 24 and 32 weeks' gestation, although not so rapidly as earlier in pregnancy. We observed rising maternal serum AFP levels until 32 weeks' gestation, consistent with two previous studies,[10, 11] but not with another study that shows a plateau early in the third trimester (28 weeks' gestation).[12] Although maternal serum AFP levels are obviously more variable in the third trimeser than in the second trimester, third-trimester variance seems not so large as originally reported.[10, 11] (SD rather than standard error is the appropriate mea-

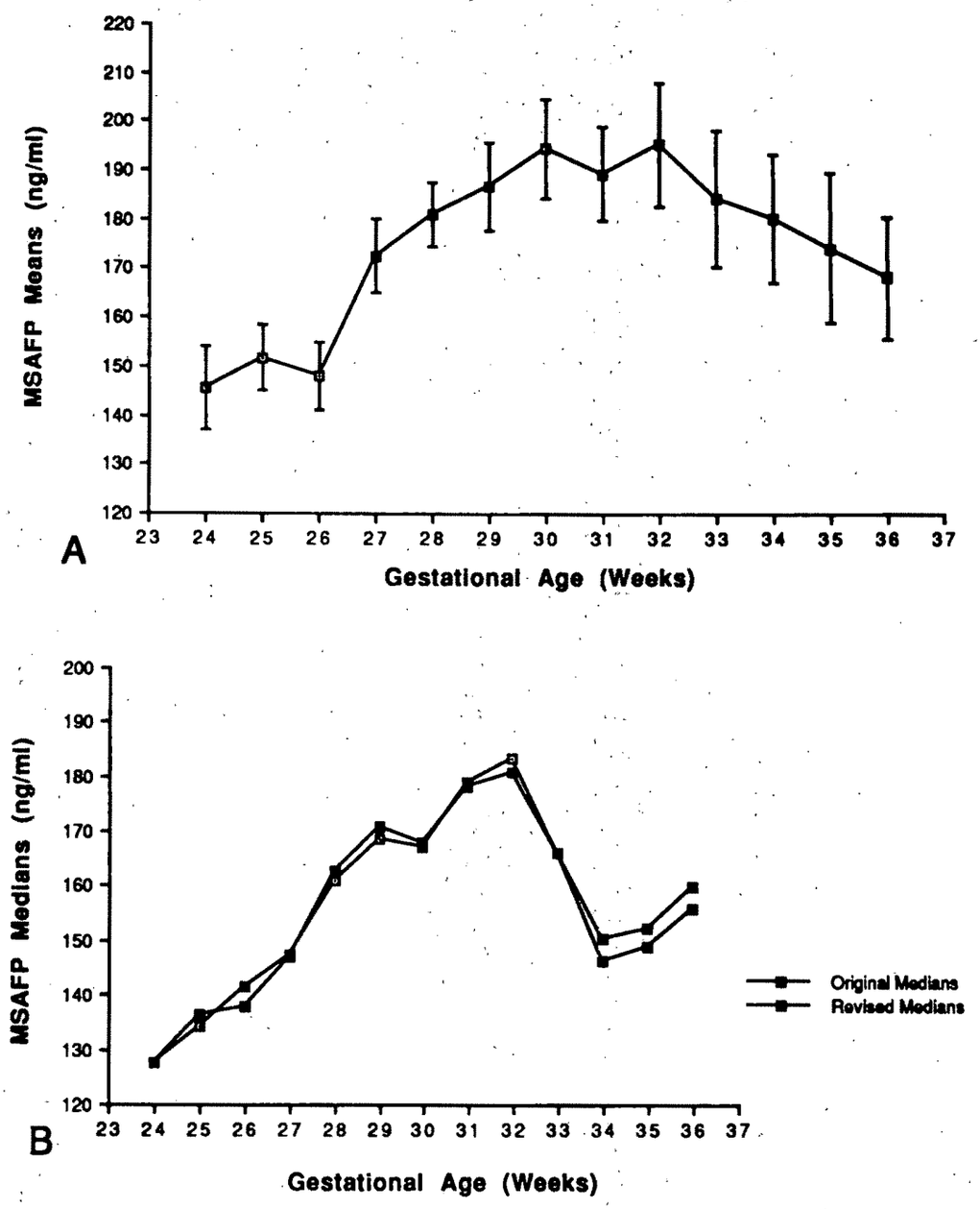


Fig. 1. Maternal serum AFP (MSAFP) values between 24 to 36 weeks' gestation. **A,** Means (±SE). **B,** Medians with and without gestational age correction.

sure because maternal serum AFP values are not normally distributed.) Second-trimester (15 to 20 weeks' gestation) SDs on a comparable number of samples in our laboratory were approximately 30 to 40 ng/ml, compared with 60 to 70 ng/ml for the late second-trimester and early third-trimester (24 to 36 weeks' gestation). We concluded that the magnitude of the variance was not so great as to preclude maternal serum AFP being clinically useful in the third trimester.

Correlation between maternal serum AFP in second and third trimester. Encouraged by the ability to assay maternal serum AFP reproducibly at 24 to 36 weeks' gestation, we next sought to determine the correlation among levels at various stages of gestation with samples obtained in the process of generating the above data.

In 354 women a single maternal serum AFP value at 15 to 20 weeks' gestation and a single value at 24 to 36 weeks' gestation were available (Table I). Most (270 of 279 or 96.8%) women with a normal (0.4 to 2.49 multiples of the median) second-trimester value showed

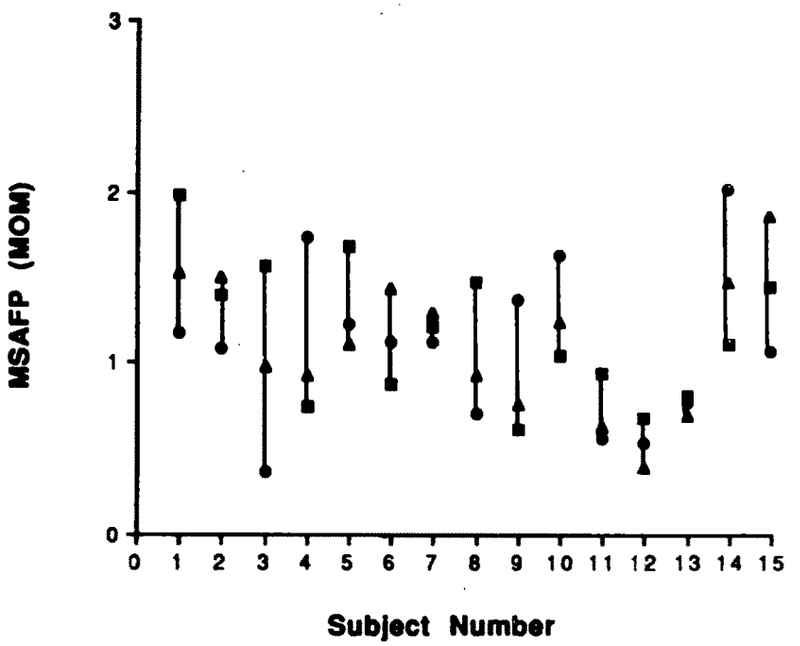


Fig. 2. Serial maternal serum AFP (MSAFP) in 15 females with three samples throughout gestation. Relationship of the three samples in individual subjects is shown.

Table I. Longitudinal studies of 286 women with two maternal serum AFP values

Second-trimester maternal serum AFP values	*Third-trimester maternal values*	
	0.4 to 2.49 MOM	*>2.49 MOM*
0.4-2.49 MOM	270	9
>2.49 MOM	7	0

MOM, Multiples of the median.

maternal serum AFP in a similar range (0.4 to 2.4 multiples of the median) later in gestation (24 to 36 weeks). This is explored further in Fig. 2, which shows serial values in 15 women with three or more values. On the other hand, none of six women with singleton pregnancies and unexplained second-trimester maternal serum AFP elevations (>2.50 multiples of the median) showed late second-trimester and early third-trimester values in this range, even though two of the six actually had an adverse perinatal outcome (Table II). Of the 24 subjects studied in the original investigation who had a late gestation maternal serum AFP level > 2.5 multiples of the median, values had been obtained in the late second-trimester or early third-trimester in nine; all nine showed levels between 0.40 and 2.49 multiples of the median. Two of the 24 experienced a fetal death and several others had abnormal outcomes (Table III). The possibility that both routine second-trimester (15 to 20 weeks) and late second-trimester and early third-trimester (24 to 36 weeks) maternal serum AFP levels have independent positive predictive value needs to be explored.

Comment

Unexplained second-trimester maternal serum AFP elevations. In the 1977 United Kingdom Collaborative Study, Wald et al.[1] reported the mean birth weight to be 281 gm lower in pregnancies associated with unexplained elevated (>2.0 multiples of the median) maternal serum AFP levels than in pregnancies associated with normal second-trimester levels. Levels > 2.0 multiples of the median were observed in 15.8% of stillbirths, 14.8% of neonatal deaths, and 16.3% of low-birth weight (<1500 gm) infants. Only 5.8% of normal singleton pregnancies showed maternal serum AFP levels > 2.0 multiples of the median. In North Carolina, Burton et al.[5,6] reported that patients with unexplained elevations (>2.5 multiples of the median) exhibited a significantly increased frequency of pregnancy complications. The frequency of fetal losses after 20 weeks' gestation was 4.0% in women with unexplained maternal serum AFP elevations, as compared with 0.5% in those with no elevation. Increases were observed for birth weight < 2500 gm (15% vs. 7.2%), neonatal deaths (2.1% vs. 0.5%), and nonchromosomal congenital anomalies (6.2% vs. 1.4%). Similar claims concerning birth weight and other adverse outcomes have been made by others.[2,3,4]

Nelson et al.[7] studied 166 patients with unusually

Table II. Outcome of women with elevated second-trimester (15 to 20 weeks' gestation) maternal serum AFP

	Routine second trimester		*Late second trimester and early third trimester*		
Subject	*MOM (not weight adjusted)*	*MOM (weight adjusted)*	*Gestational age on sampling (wk)*	*MOM (not weight adjusted*)*	
1	2.48	2.52	24	1.79	Uncomplicated pregnancy
2	3.24	2.95	36	0.88	Uncomplicated pregnancy
3	2.75	2.93	26	1.92	Premature rupture of membranes; neonatal death
4	2.35	2.85	34	1.50	Uncomplicated pregnancy
5	4.97 (Elevated for twins)	5.70 (Elevated for twins)	24	2.60	Uncomplicated twin pregnancy
6	2.60	2.78	30	0.66	Abruptio placentae; postpartum (5 wk) eclampsia
7	2.09	2.55	26	1.23	Uncomplicated pregnancy

MOM, Multiples of the median.

*No formula for weight adjusting late second-trimester and early third-trimester values. Applying the routine second-trimester formula to such values would have resulted in maternal serum AFP of subject 5 still being ≥2.5 multiples of the median and all other subjects <2.5 multiples of the median.

high maternal serum AFP levels (>5 multiples of the median). Of these, 100 (66%) had a subsequent pregnancy complication. The complication rate was 30% if levels were 4 to 4.9 multiples of the median, 26% if levels were 3 to 3.9 multiples of the median, and 14% if levels were 2.5 to 2.9 multiples of the median. A correlation between frequency of complications and degree of maternal serum AFP elevation was partially confirmed by Robinson et al.[13] From an initial sample of 35,787 women, 560 with unexplained maternal serum AFP > 2.5 multiples of the median were identified. The frequency of stillbirths increased as maternal serum AFP levels increased, although this did not hold for intrauterine growth retardation.

With respect to pregnancy complications other than fetal death or growth retardation, Clayton-Hopkins et al.[14] reported that maternal serum AFP values were elevated in pregnancies complicated by hypertension. This finding was also observed by Mizejewski and Risemberg.[15] In a case-control study by Milunsky et al.[16] women whose pregnancies were characterized by obstetric complications were matched with women whose pregnancies had normal outcomes. Increased odds ratios were observed between elevated second-trimester maternal serum AFP levels and the following adverse perinatal outcomes and pregnancy complications: fetal death (8.1), low birth weight (4.0), newborn complications (3.6), oligohydramnios (3.4), abruptio placentae (3.0), preeclampsia (2.3), premature rupture of membranes (2.0), and polyhydramnios (1.6). It is noteworthy that these complications are not independent of each other. Moreover, corrections for multiple comparisons were apparently not made.

Although intriguing, objections can be raised to the experimental designs of some of the above studies. Biases of ascertainment probably exist in some studies. One obvious pitfall would arise if blood samples were preferentially submitted from women already suspected of having a complication (ascertainment bias). This pitfall was especially likely to have been a problem before examination of maternal serum AFP became routine (circa 1985). If some fetuses were moribund, if in fact not already dead, maternal serum AFP levels might merely be secondarily elevated. Misclassification of an inviable pregnancy as viable would contribute to an apparent association between maternal serum AFP elevation and fetal death and possibly other complications. If the purported association were applied clinically to women known to have viable pregnancies at the time of screening, risks would be overestimated. Burton et al.[2, 3, 7] verified fetal viability at the time of maternal serum AFP screening. The assumption of fetal viability is also plausible in the case-control study of Milunsky et al.,[16] because this study was conducted relatively recently, at a time of high use of ultrasonography. However, viability at the time of sampling cannot be assumed in all studies. One could also envision circumstances in which unexplained second-trimester maternal serum AFP elevations would carry no significance for untoward outcome because the pathogenesis for the elevation would be transient. For example, maternal-fetal hemorrhage could lead to a rise in maternal serum AFP level. If no further bleeding occurred, perinatal outcome would not necessarily be unfavorable. Another circumstance in which transient maternal serum AFP elevation is not necessarily associated with adverse clinical consequence is that in which resorption of a dead co-twin occurs.

Finally, it is uncertain whether unexplained maternal serum AFP elevation is predictive of complications in

Table III. Outcome in 24 women with elevated late second-trimester and early third-trimester (24 to 36 weeks' gestation) maternal serum AFP levels

	Routine second trimester		*Late second trimester and early third trimester*	
Subject	*MOM (not weight adjusted)*	*MOM (weight adjusted)*	*Gestational age on sampling (wk)*	*MOM (not weight adjusted*)*
1	—	—	32	2.55 Postdate pregnancy (42 wk); normal outcome
2	—	—	29	3.53 Meconium stained amniotic fluid at 36 wk
3	3.39	3.82 normal for twins	35	2.65 Uncomplicated twin pregnancy; cesarean section
4	—	—	33	2.48 Meconium-stained amniotic fluid at 39 wk
5	4.97	5.70 (elevated for twins)	24	3.24 Uncomplicated twin pregnancy; cesarean section
6	1.17	1.48	31	2.68 Preterm labor at 32 wk; pregnancy-induced hypertension; delivery 36 wk
7	—	—	26	2.55 Meconium-stained amniotic fluid; cesarean section for fetal distress at 41 wk
8	—	—	29	2.60 Cesarean section for breech presentation; subchorionic hematoma
9	1.28	1.28	32	2.45 Fetal death at 35 wk (severe maceration at birth); subchorionic hematoma
10	2.14	1.71	30	2.49 Uncomplicated pregnancy
11	—	—	34	2.47 No delivery data available
12	—	—	27	3.01 Twin pregnancy; delivery at 30 wk
13	1.16	1.32	30	2.63 Meconium-stained amniotic fluid at 42 wk
14	—	—	33	2.76 Pregnancy-induced hypertension; meconium-stained amniotic fluid at 40 wk; repeat cesarean section
15	1.28	1.22	36	2.93 Uncomplicated pregnancy
16	—	—	24	6.30 Fetal death next day; no fetal heart tones day of sample
17	—	—	34	2.50 Meconium-stained amniotic fluid at 41 wk
18	1.20	1.56	34	2.69 Uncomplicated pregnancy
19	1.64	1.56	36	2.72 Uncomplicated pregnancy
20	—	—	28	2.50 Uncomplicated pregnancy
			35	4.04 First-trimester chorionic villus sampling
21	—	—	33	2.47 Uncomplicated pregnancy
22	—	—	27	2.82 Uncomplicated pregnancy
23	—	—	27	2.99 Postdates pregnancy (42 wk); normal outcome
24	—	—	36	3.08 Uncomplicated pregnancy

MOM, Multiples of the median.

*No formula for weight adjusting late second-trimester and early third-trimester values. Application of the routine second-trimester formula to these values would have resulted in maternal serum AFP of subjects 7, 10, and 17 falling to <2.5 multiples of the median.

women not otherwise suspected of having obstetric problems. Perhaps unexplained elevation merely confirms pregnancies already recognized clinically to be at increased risk as a result of pregnancy complications such as preeclampsia, preexisting maternal illnesses (e.g., diabetes mellitus), bleeding, or complications in a previous pregnancy. If so, unexplained maternal serum AFP elevation would add little to what the alert obstetrician already knows. Clearly data are needed that state the predictive value of elevated maternal serum AFP levels in women manifesting no known risk factors who are known to have a viable pregnancy.

In conclusion, data seem insufficient to justify aggressive surveillance in pregnancies of otherwise nor-

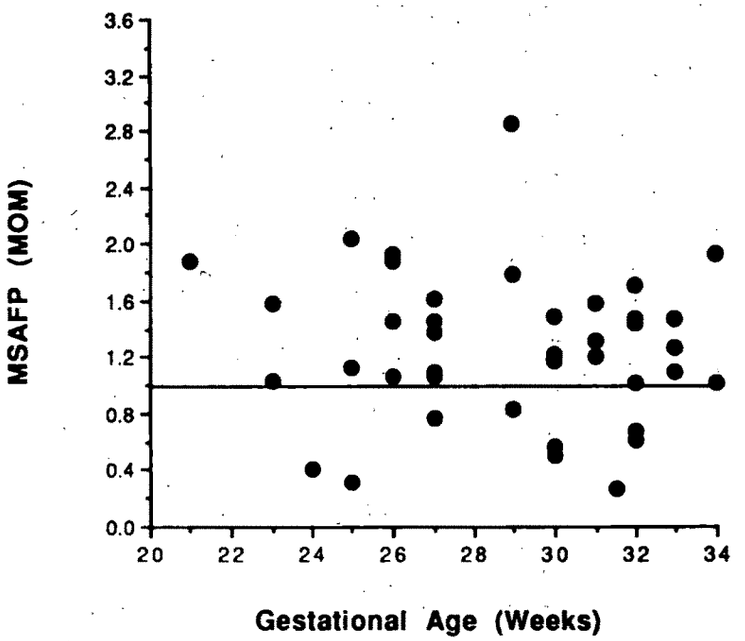


Fig. 3. Maternal serum AFP (MSAFP) in women with premature rupture of membranes not yet in labor.

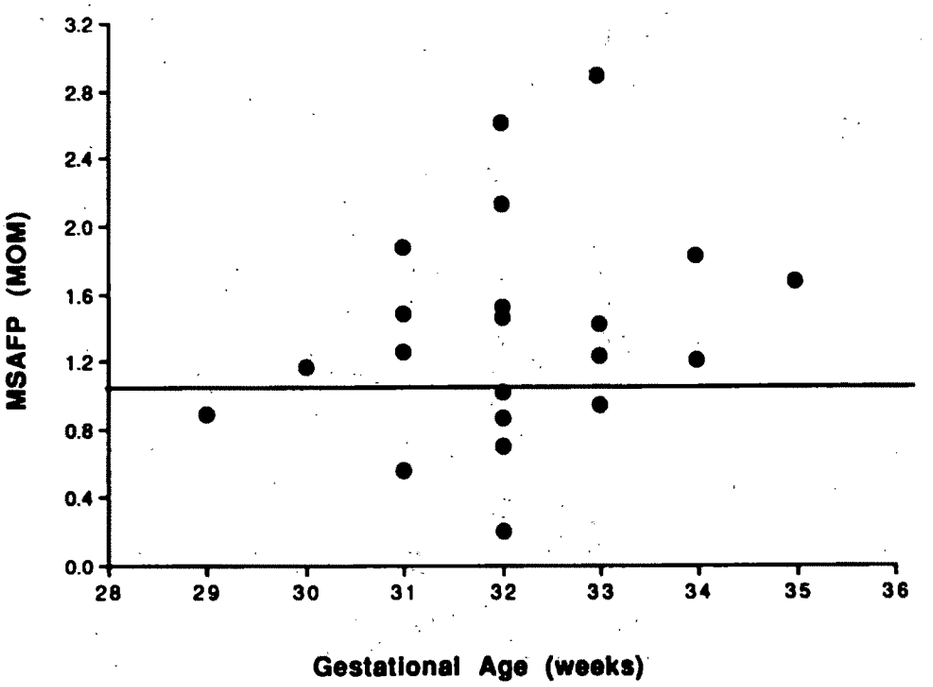


Fig. 4. Maternal serum AFP (MSAFP) values in women with preterm labor subsequently delivered before 37 weeks' gestation.

mal women who show unexplained second-trimester maternal serum AFP elevations. Although the prudent obstetrician will exercise due care and oversight, we believe it is premature to counsel such women that they are at increased risk of poor pregnancy outcomes such as perinatal death or low birth weight.

Third-trimester maternal serum AFP. Third-trimester maternal serum AFP assays were pursued over a decade ago but apparently were abandoned because of discrepant results. Our data indeed confirm that maternal serum AFP levels in the third trimester show greater variance than those in the early second

trimester. However, the variance is not so great that usefulness of third-trimester screening is precluded. Table III further suggests that late second-trimester and early third-trimester maternal serum AFP elevations could be predictive of pregnancy complications. Two of 24 pregnancies characterized by elevations showed fetal death; several other pregnancies showed other obstetric complications. In aggregate, Tables II and III suggest that maternal serum AFP elevations in both the second and third trimesters are useful yet independent of one another.

One way to verify the value of unexplained second-trimester elevations is by longitudinal analysis of maternal serum AFP later in pregnancy. One would predict that unexplained routine second-trimester (15 to 20 weeks' gestation) elevations in otherwise normal women would remain elevated during the late second-trimester and early third-trimester in those pregnancies manifesting an adverse perinatal outcome. If not, previous claims of a relationship between second-trimester elevations and adverse perinatal outcome might either be specious or merely confirmatory of information already known clinically. On the other hand, one could postulate that in certain conditions maternal serum AFP might be elevated in the second trimester but more nearly normal later in gestation as a result of changing placentation. Moreover, the test may be useful only during the time of gestation during which maternal serum AFP was rising (i.e., before 32 weeks' gestation).

Two approaches can help determine the significance of elevated second-trimester maternal serum AFP levels and the potential use of late second-trimester and early third-trimester levels. First, large scale longitudinal studies throughout pregnancy are obviously necessary. Second, cross-sectional studies should be pursued. Indeed, a cross-sectional approach is the basis for routine second-trimester (15 to 20 weeks' gestation) maternal serum AFP screening, and pragmatically would be the approach used clinically later in pregnancy. In Fig. 3 there is a tendency for women with preterm premature rupture of membranes, not yet manifesting labor, to show maternal serum AFP levels above the median. Fig. 4 shows an apparent association between elevated maternal serum AFP levels at 24 to 36 weeks' gestation and premature labor. Confirmation of these trends would suggest a diagnostic role for maternal serum AFP in the third trimester.

In conclusion, the value of unexplained second-trimester maternal serum AFP elevations in identification of pregnancies at risk of adverse outcomes other than neural tube defects seems worth pursuing. However, important issues are not being addressed, namely the extent to which ascertainment biases influence extant data, and the extent to which elevated maternal serum AFP levels contribute information different from that already known by clinically alert obstetricians. In our opinion, until these issues are addressed, aggressive (and expensive) perinatal surveillance programs should not be recommended for otherwise normal women with unexplained second-trimester (15 to 20 weeks' gestation) maternal serum AFP elevations. We propose that this issue can be resolved by determining the relationship between second- and third-trimester maternal serum AFP levels. Our preliminary data further indicate that late second-trimester and early third-trimester levels could be useful in identification of women with certain pregnancy complications.

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Cardiac function in fetuses of type I diabetic mothers

Giuseppe Rizzo, MD,[a] Domenico Arduini, MD,[a] and Carlo Romanini, MD[b]
Rome and Ancona, Italy

Cardiac function was cross-sectionally studied by means of M-mode and Doppler echocardiography in 40 fetuses of mothers with well-controlled insulin-dependent diabetes at 20 to 38 weeks of gestation. These variables were measured: interventricular septal thickness, ratio between the peak velocities during early passive ventricular filling and active atrial filling at the level of the atrioventricular valves, peak velocities, and the time to peak velocity at the level of the ascending aorta and the pulmonary artery. The values obtained were compared with our reference limits for gestation. A significant increase of interventricular septal thickness that was unrelated to maternal glycosylated hemoglobin levels was evidenced. Early passive ventricular filling/active atrial filling ratios were significantly lower in fetuses of diabetic mothers than in control fetuses. These differences were significantly related to interventricular septal thickness. No significant modifications were found in either aortic or pulmonary peak velocities or in time to peak velocity values. These findings suggest that in spite of an adequate metabolic control an interventricular septal hypertrophy that affects cardiac diastolic function develops in fetuses of diabetic mothers. (AM J OBSTET GYNECOL 1991;164:837-43.)

Key words: Diabetes, fetal heart, echocardiography, Doppler ultrasonography, diastolic function

Although the existence of hypertrophic cardiomyopathy in infants of diabetic mothers has been extensively described,[1] few data currently are available on the development of this anomaly during intrauterine life.[2, 3] In this study M-mode and Doppler echocardiography was used to study cardiac function in fetuses of mothers with type I diabetes.

Material and methods

Patients. Forty-two women with type I insulin-dependent diabetes were evaluated in this study. Twenty-four women belonged to class B, 10 to class C, and 8 to class D, according to the modified White's classification.[4] All pregnancies were singleton and were accurately dated by early ultrasonographic examination. Diabetes was controlled by self-monitoring of blood glucose levels, and insulin adjustments were performed at the biweekly visit, if necessary, with the goal of maintaining the preprandial and postprandial blood glucose levels below 90 and 120 mg/dl, respectively. The obstetric characteristics of the patients are reported in Table I. At the time of echocardiographic examination the glycosylated hemoglobin (HbA_{1C}) was measured, and the results obtained were correlated with the echocardiographic findings. All patients gave informed consent to participate in the study. Each patient was included in the study only once.

Table I. Characteristics of 40 patients with type 1 diabetes considered for study

Age (yr, mean ± SD and range)	24.9 ± 4.4	(19-35)
Parity (mean ± SD and range)	1.3 ± 0.5	(0-4)
HbA_{1C} in first trimester (%, mean ± SD and range)	6.0 ± 1.0	(4.8-7.4)
HbA_{1C} at Doppler recording	5.7 ± 0.8	(4.5-6.9)
Gestational age at Doppler recording (wk, mean ± SD and range)	29.7 ± 4.8	(20-38)
Gestational age at delivery (wk, mean ± SD and range)	38.3 ± 1.9	(35-40)
Birth weight (gm, mean ± SD and range)	3353 ± 576	(2640-4220)
Large-for-gestational-age newborns (>90th percentile)	4 (10%)	
Fetal distress necessitating emergency cesarean section	9 (22.5%)	

Echocardiographic examination. Commercially available Ansaldo Hitachi Esacord 81 (Genoa) color Doppler equipment with 3.5 or 5 MHz convex probes was used for all measurements. The machine is equipped with a color flow mapping function, as well

From the Laboratory of Fetal Physiology, Department of Obstetrics and Gynecology, Università Cattolica S. Cuore, Rome,[a] and the Department of Obstetrics and Gynecology, Università di Ancona.[b]
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Reprint requests: G. Rizzo, MD, Ist. Cl. Ostetrica e Ginecologica, Università Cattolica S. Cuore, Largo A. Gemelli, 8, 00168 Roma, Italy.
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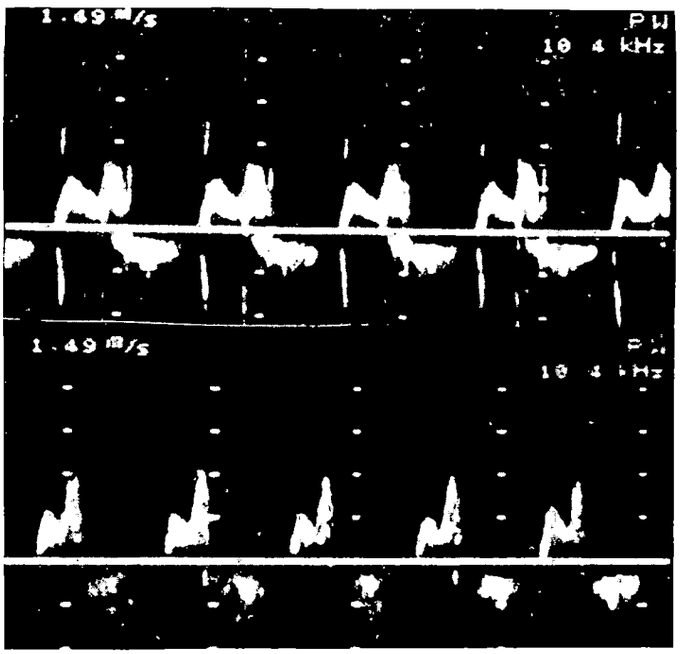


Fig. 1. Flow velocity waveforms through mitral valve in a control fetus *(top)* and in a fetus of a diabetic mother *(bottom)* at 31 weeks of gestation. E/A ratio values are 0.72 in the former and 0.44 in the latter.

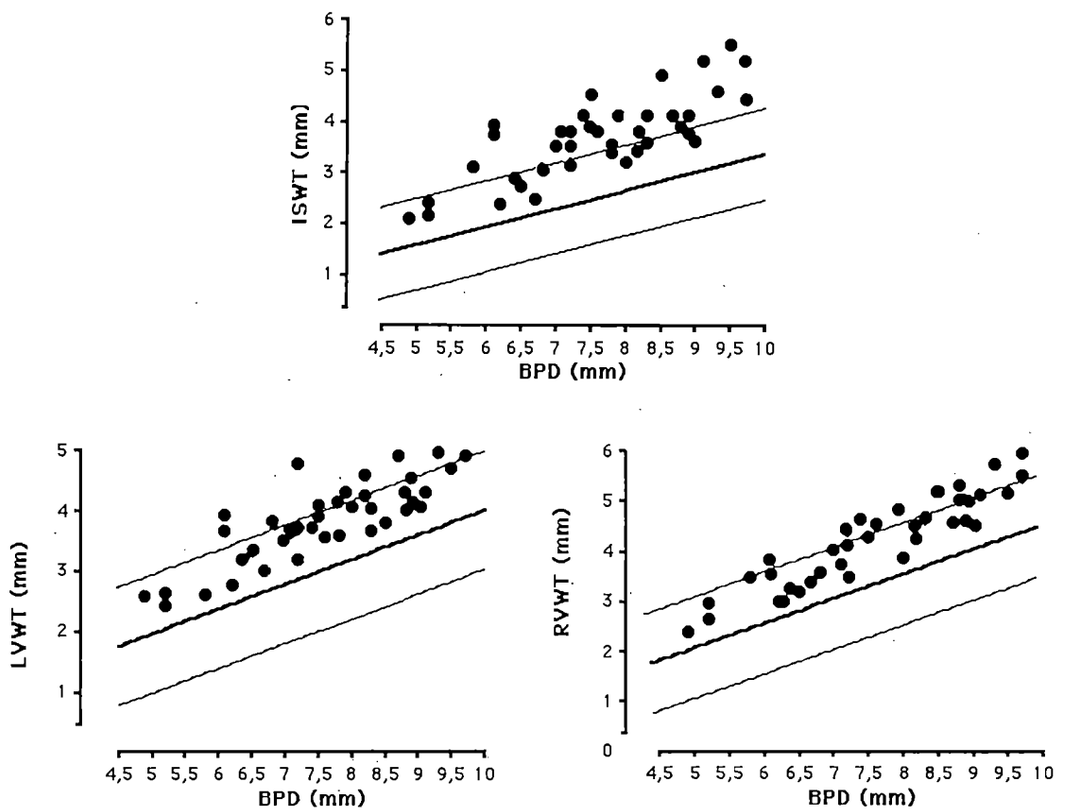


Fig. 2. ISWT, LVWT, and RVWT values obtained in fetuses of diabetic mothers, as plotted on our reference range (mean and 95% confidence intervals).

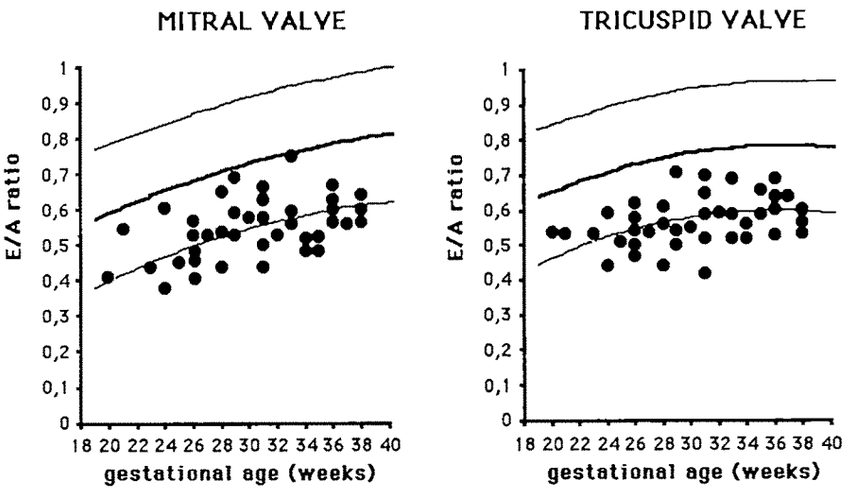


Fig. 3. Transtricuspid and transmitral E/A ratios obtained in fetuses of diabetic mothers, as plotted on our reference ranges for gestation (mean and 95% confidence intervals).

as a pulsed Doppler ultrasonography with a carrier frequency that ranges from 2.5 to 5 MHz.

The fetal heart was first examined with two-dimensional echocardiography, and two patients were excluded from the study because of congenital heart diseases (i.e., tetralogy of Fallot and atrioventricular canal). A subcostal four-chamber view of the fetal heart was obtained in the remaining 40 patients. The M-mode beam was directed perpendicular to the interventricular septum at the level of the atrioventricular valves, and images were recorded.[5] Velocity waveforms were then recorded at the level of the atrioventricular valves, ascending aorta, and pulmonary artery, according to previously reported techniques.[6, 7] Mitral and tricuspid valves were visualized from the apical four-chamber view, as were the aortic valve from the five-chamber view and the pulmonary valve from the short-axis view. The color flow mapping function was then superimposed, and the valve flow was evidenced. Care was taken to visualize the flow so that it was parallel to the Doppler beam. The pulsed Doppler sample volume was placed immediately distal to valve leaflets, and velocity waveforms were recorded. Recordings at a beam angle larger than 20 degrees were rejected.

Doppler and M-mode images were recorded on standard ½-inch videotape for subsequent analysis. Permanent records were obtained from the videotape by means of a strip chart recorder. Ten consecutive heart cycles were selected during periods of fetal rest without breathing movements, and the values measured were averaged. These variables were measured with the aid of a computer-interfaced digitizer pad (Cardio 800, Kontron, Oxford): (1) wall thickness during end diastole at the level of the interventricular septum (ISWT), left ventricle (LVWT), and right ventricle (RVWT)[5]; (2) tricuspid and mitral blood flow velocities during early ventricular filling (E wave) and during atrial systole (A wave)[6]; (3) the peak velocities of the aortic and pulmonary valves; (4) pulmonary and aortic time to peak velocity values, which were calculated as the time differences from the onset of the waveform to its peak velocity[7]; (5) heart rate. The ratios between E-wave and A-wave peak velocities (the E/A ratio) at the level of both atrioventricular valves were then calculated as indices of ventricular diastolic function.[6]

Data analysis. The variables measured were compared with our reference limits that were obtained from the cross-sectional study of 197 fetuses from normal pregnancies that were between 20 and 40 weeks' gestation. Because the size of the heart is better related to fetal dimension than to gestational age,[5] reference limits for ISWT, LVWT, and RVWT were constructed considering the fetal biparietal diameter as the independent variable. In addition, gestational age was used as the independent variable to calculate normative data for Doppler measurements.[6, 7]

Because all the variables considered change with gestation, the individual echocardiographic values observed in fetuses of diabetic mothers were also expressed as the number of standard deviations from the respective normal means for gestation (e.g., ΔISWT, Δmitral E/A ratio). A one-sample Student *t* test was used to compare echocardiographic parameters of control fetuses with those of fetuses of diabetic mothers, whereas the differences among diabetic fetuses were tested with the unpaired Student *t* test. Simple and multiple regression analyses were used to test the relationships between the variables considered. These analyses were performed on a Macintosh II (Apple Computers, Cupertino, Calif.) computer with the Stat View II statistical package (Abacus Concepts Inc., Berkeley, Calif.).

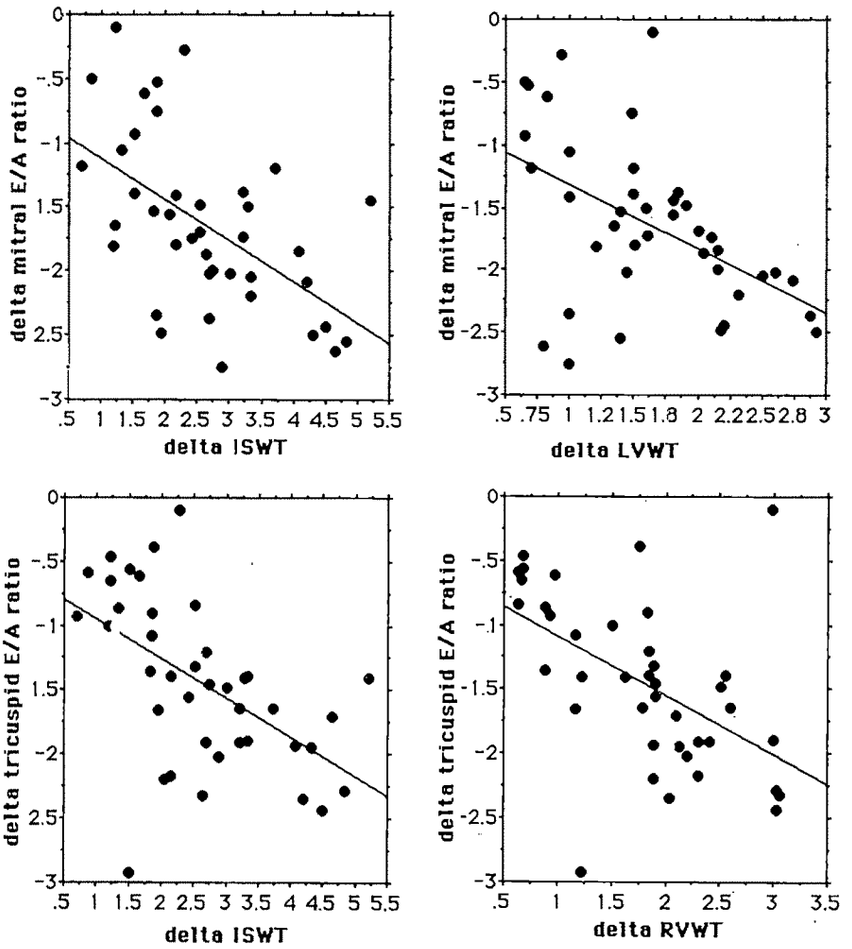


Fig. 4. Relationships between mitral E/A ratio with ISWT *(left)* and LVWT *(right)* and between tricuspid E/A ratio with ISWT *(left)* and RVWT *(right)*. All values are expressed as number of SDs by which they differed from the respective means for gestational age (Δ-values).

Results

Successful recordings were obtained in all cases at the level of the atrioventricular valves (Fig. 1). In 32 cases reliable data were obtained from all four cardiac sites, whereas fetal position did not allow successful recordings at the level of the pulmonary artery and ascending aorta, respectively, in three and five cases. At birth none of the infants had clinical signs of cardiomyopathy, with the exception of the two fetuses excluded for congenital heart diseases.

M-mode echocardiography. The ISWT values of fetuses of diabetic mothers were significantly higher (mean difference 2.632 SDs, $t = 16.437$, $p \leq 0.001$) when compared with our reference limits for gestation. Similar results were obtained when LVWT (mean difference 1.626 SDs, $t = 15.987$, $p \leq 0.001$) and RVWT (mean difference 1.790 SDs, $t = 15.201$, $p \leq 0.001$) values were considered (Fig. 2). However, there were no relationships between HbA_{1c} and ΔISWT ($r = 0.194$), ΔLVWT ($r = 0.123$), and ΔRVWT ($r = 0.106$) values. Furthermore no differences were found in M-mode parameters when fetuses of diabetic mothers assigned to White's class B were compared with those fetuses of mothers in classes C and D.

Diastolic function. In the fetuses of diabetic mothers the E/A ratio results were significantly lower at the level of both mitral valves (mean difference −1.634 SDs, $t = 15.414$, $p \leq 0.001$) and tricuspid valves (mean difference −1.449 SDs, $t = 14.038$, $p \leq 0.001$) when compared with the E/A ratios of control fetuses (Fig. 3). A significant negative relationship was found between Δ mitral E/A ratio and both ΔISWT (constant = −0.790, slope = −0.321, SD = 0.567, $r = 0.552$, $p \leq 0.001$) and ΔLVWT values (constant = −0.794, slope = −0.517, SD = 0.590, $r = 0.496$, $p \leq 0.002$) (Fig. 4) with a multiple r value of 0.601. Simi-

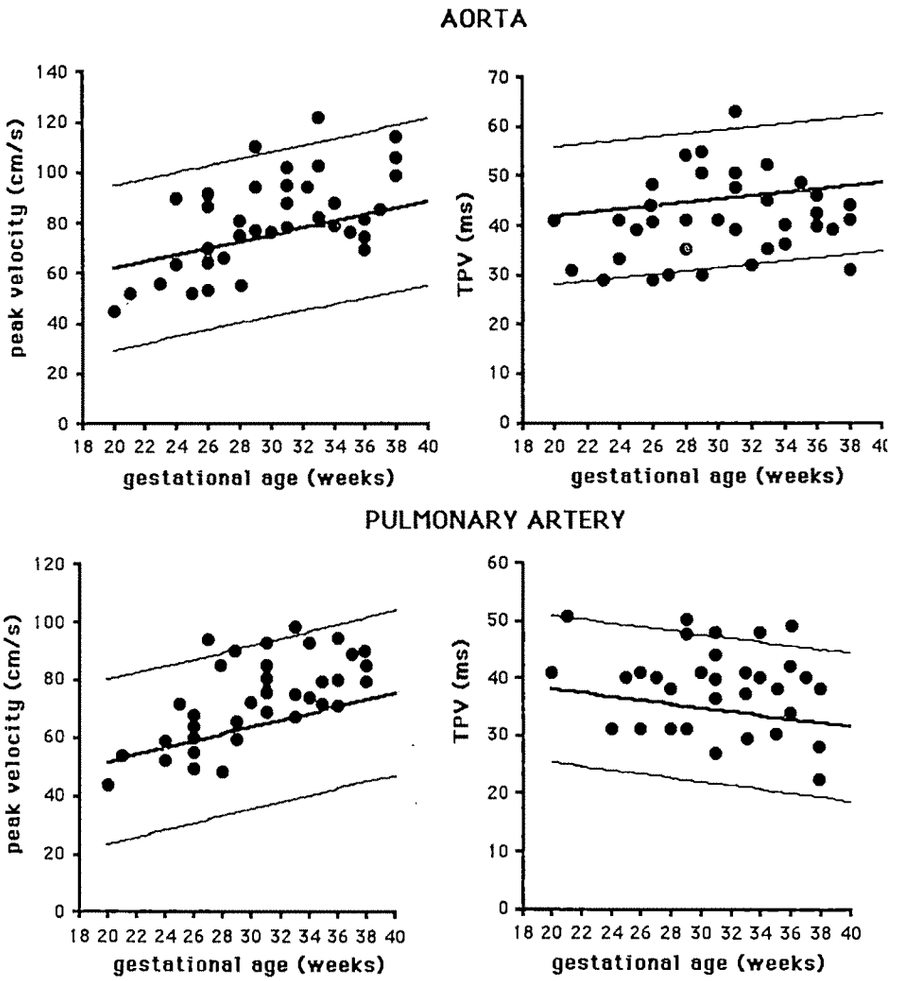


Fig. 5. Aortic *(top)* and pulmonary *(bottom)* peak velocity and time to peak velocity *(TPV)* values in fetuses of diabetic mothers, as plotted on our reference ranges for gestation (mean and 95% confidence intervals).

larly, Δ tricuspid E/A ratio results were negatively related to ΔISWT (constant = −0.643, slope = −0.306, SD = 0.556, $r = 0.541$, $p \leq 0.002$) and ΔRVWT values (constant = −0.629, slope = −0.458, SD = 0.564, $r = 0.491$, $p \leq 0.005$) (Fig. 4) with a multiple r value of 0.590. The HbA_{1c} value results were unrelated to mitral ($r = 0.141$) and tricuspid ($r = 0.152$) Δ E/A ratios. No significant differences were found in E/A ratios when fetuses were grouped according to White's class of the mother.

Systolic function. In Fig. 5 aortic and pulmonary values of peak velocity and time to peak velocity obtained in fetuses of diabetic mothers are plotted against our reference limits for gestation. No significant changes were evidenced and no significant correlations were found with ΔISWT, ΔRVWT, and ΔLVWT ($r < 0.116$), with HbA_{1c} ($r < 0.123$) values, with White's class of the mother.

Relations to pregnancy outcome. Nine fetuses required emergency cesarean section because of fetal distress (abnormal fetal heart patterns characterized by late or repetitive variable decelerations and/or loss of baseline variability) (Table I). When these fetuses were compared with the remaining fetuses of diabetic mothers studied, no significant differences were found in the cardiac parameters considered (Table II). Similarly, there were no differences when echocardiographic findings of large-for-gestational age fetuses (i.e., birth weight above the 90th percentile) were compared with those of appropriate-for-gestational-age fetuses (Table II).

Comment

A linear increase with fetal growth of both heart size and wall thickness has been evidenced in the human fetus.[5] Moreover, a concomitant improvement of the

Table II. Echocardiographic measurements in fetuses of diabetic mothers grouped according to birth weight or occurrence of fetal distress (i.e., abnormal heart rate patterns necessitating emergency cesarean section)

	Fetal distress (No. = 9)	*Remaining fetuses (No. = 31)*	*p Value**	*LGA fetuses (No. = 4)*	*AGA fetuses (No. = 36)*	*p Value**
ISWT	2.66 ± 1.29	2.62 ± 1.14	NS	2.52 ± 1.44	2.60 ± 1.28	NS
LVWT	1.65 ± 0.67	1.62 ± 0.63	NS	1.66 ± 0.90	1.63 ± 0.62	NS
RVWT	1.76 ± 0.78	1.80 ± 0.74	NS	1.82 ± 0.75	1.79 ± 0.74	NS
Mitral E/A ratio	−1.59 ± 0.74	−1.66 ± 0.67	NS	−1.62 ± 0.72	−1.65 ± 0.63	NS
Tricuspid E/A ratio	−1.54 ± 0.69	−1.46 ± 0.63	NS	−1.50 ± 0.67	−1.48 ± 0.64	NS
Peak velocity, aorta	0.33 ± 0.71	0.45 ± 0.44	NS	0.40 ± 0.88	0.47 ± 0.39	NS
Time to peak velocity, aorta	−0.21 ± 0.46	−0.34 ± 0.39	NS	−0.59 ± 0.62	−0.31 ± 0.33	NS
Peak velocity, pulmonary artery	0.87 ± 0.67	0.79 ± 0.47	NS	0.73 ± 0.69	0.75 ± 0.44	NS
Time to peak velocity, pulmonary artery	−0.18 ± 0.33	0.16 ± 0.39	NS	0.22 ± 0.33	0.14 ± 0.31	NS

LGA, Large for gestational age; *AGA*, appropriate for gestational age. All values are expressed as number of standard deviations by which they differed from the respective means for gestational age (Δ-values).

* Unpaired *t* test.

cardiac performance has been described. The E/A ratio, a widely accepted index of ventricular diastolic function,[8] significantly increases during gestation at the level of both atrioventricular valves. These changes are believed to be secondary to the progressive maturation of ventricular compliance.[9] There is also an increase of aortic and pulmonary peak velocities and of left and right stroke volumes,[10] which suggests an improvement of ventricular systolic function with gestation. Previous studies have shown that in infants of diabetic mothers there is a hypertrophic cardiomyopathy that is characterized primarily by a significant thickening of the interventricular septum.[1, 11] Our data on a large series confirm previous sporadic reports[2, 3] that the increase of ISWT, LVWT, and RVWT is already established during intrauterine life. The absence of correlations between the HbA_{1c} values and the severity of cardiac wall thickness found in this investigation is in agreement with the results of neonatal studies[12] and suggests that in spite of adequate metabolic control during pregnancy, fetuses of diabetic mothers might experience an abnormal cardiac growth. However, there is no current account regarding whether the increase of cardiac wall thickness merely reflects the larger size of fetuses of diabetic mothers or whether it might also influence the fetal cardiac function. The lower E/A ratios at the level of both atrioventricular valves found in these fetuses might indicate an impaired ventricular diastolic function. The inverse relationships between the E/A values and both the interventricular septum and lateral ventricular wall thickness suggest that the abnormal cardiac thickening might impair ventricular compliance, which would affect the filling patterns of the ventricles. Previous microscopic studies on the heart in newborns of diabetic mothers corroborate this hypothesis by showing a disorganization of the myofibrils, which is similar to that present in adults with hypertrophic cardiomyopathy,[1, 13] a condition characterized by evident alterations of ventricular compliance and thus of Doppler-measured diastolic function.[14]

In spite of a ventricular hypercontractility reported in infants of diabetic mothers,[1] no significant modifications of systolic indices such as aortic and pulmonary peak velocities or time to peak velocity were evidenced in the fetuses examined. In adults diastolic dysfunction usually precedes systolic anomalies that appear later in the development of the myocardial disease.[15] Therefore it can be speculated that the fetuses studied have not reached a degree of cardiac impairment sufficient to affect systolic function. This hypothesis is supported by the data available on infants of diabetic mothers which show that echocardiographic indices of systolic function fall either in the normal range or above the normal range[1] and are usually abnormal only in newborns with clinical signs of cardiac impairment.[16]

In conclusion our data suggest that despite careful metabolic control of pregnancy, fetuses of diabetic mothers show an increased thickness of the interventricular septum and of the ventricular walls, which may be associated with abnormalities of diastolic function. Further longitudinal studies during the perinatal period are required to clarify the clinical significance of these changes of cardiac function and their sequelae during neonatal life.

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Human placenta expresses endothelin gene and corresponding protein is excreted in urine in increasing amounts during normal pregnancy

Ariela Benigni, Biol.Sci.D.,[a] Flavio Gaspari, Chem.D.,[a] Silvia Orisio, Biol.Sci.D.,[a] Luca Bellizzi, Chem.,[a] Giuseppe Amuso, MD,[b] Tiziana Frusca, MD,[d] and Giuseppe Remuzzi, MD[a, c]

Bergamo and Brescia, Italy

Systemic and renal hemodynamic changes occurring in normal pregnancy have been attributed in part to altered vascular synthesis of vasodilatory prostaglandins. Besides vasodilatory substances, endothelium also generates vasoconstrictors, including endothelin. We evaluated the capacity of placental tissue from normal pregnant women to express endothelin gene and to generate endothelin. Placental tissue expressed a single 2.3 kb preproendothelin messenger ribonucleic acid and produced comparable amounts of endothelin 3, Big endothelin 1, and endothelin 1 and a minor quantity of endothelin 2. To investigate the possible influence of placental endothelin production on plasma levels of the peptide, plasma endothelin concentrations were measured in normal pregnant women at delivery and were found to be numerically higher than those measured in nonpregnant subjects. Urinary excretion of endothelin, taken as a marker of the renal synthesis of the peptide, tended to increase, although not significantly, in the first 14 weeks of pregnancy. This trend continued throughout pregnancy, resulting in a significant increase from the second trimester to delivery. (AM J OBSTET GYNECOL 1991;164:844-8.)

Key words: Endothelin gene expression in placenta, placenta endothelin production, plasma endothelin, endothelin urinary excretion in normal pregnancy

Normal pregnancy is associated with characteristic changes in systemic and renal hemodynamics. A substantial decrease in peripheral vascular resistance offsets increases in blood volume and cardiac output and results in a modest fall in blood pressure during the course of a normal pregnancy.[1] This change in blood pressure is maximal during the midtrimester[2] and tends to return to normal during the third trimester. Concomitant with systemic hemodynamic changes, an increase in renal plasma flow, associated with a rise in glomerular filtration rate, has been reported.[3] The intrarenal mechanisms responsible for the changes in renal plasma flow and glomerular filtration rate have been studied by micropuncture technique in the rat, which showed that the single-nephron glomerular filtration rate is increased as early as 5 to 12 days of gestation.[4] The decreased vascular tone and the modifications in renal hemodynamics occurring in pregnancy may be attributed in part to changes in the synthesis of endothelial-derived vasoactive substances. Thus in normal pregnancy systemic[5] and renal[6] synthesis of vasodilatory prostacyclin[7] is increased and may be responsible for the marked resistance to the pressor effects of angiotensin II.[8] Vascular endothelium generates vasodilators and vasoconstrictor substances, and a proper balance between vasodilators and vasoconstrictors may be crucial to the adaptive changes occurring in pregnancy. In this context the recent discovery that vascular endothelium produces endothelin, a 21 amino acid residue peptide with potent vasoconstrictor properties,[9] has prompted us to investigate whether normal placental tissue expresses the endothelin gene and generates endothelin peptides and whether endothelin is measurable in the plasma or is excreted in the urine during the course of normal pregnancy.

From the Mario Negri Institute for Pharmacological Research,[a] the 2nd Division of Obstetrics and Gynecology,[b] and the Division of Nephrology,[c] Ospedali Riuniti di Bergamo, and the Obstetrics and Gynecology Clinic, University of Brescia.[d]
Received for publication June 28, 1990; revised September 28, 1990; accepted October 15, 1990.
Reprint requests: Giuseppe Remuzzi, MD, Mario Negri Institute for Pharmacological Research, Via Gavazzeni, 11, 24100 Bergamo, Italy.
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Material and methods

Study groups consisted of women attending the Obstetric Divisions of Ospedali Riuniti in Bergamo and Spedali Civili in Brescia. All subjects denied taking any drug for at least 14 days before the study.

Three groups of normotensive women were studied: Group 1 consisted of nine women in the first trimester

(0 to 14 weeks), group 2 was made up of seven women in the second trimester (14 to 28 weeks), and group 3 contained eight women admitted for routine delivery at 28 to 40 weeks' gestation. All women had blood pressure not exceeding 140/85 mm Hg, did not have significant proteinuria, and gave birth to a live infant of normal weight after 36 weeks' gestation. The placenta appeared normal in all cases. An additional group of nine nonpregnant women from the staff of the two divisions was studied as a control group.

Term placentas from five of the pregnant women were obtained at delivery and processed within 30 minutes from collection. A mixture of vaginal ($n = 3$) and cesarean section ($n = 2$) deliveries was studied. Placental tissues were separated, gently cut into small pieces, and washed repeatedly with phosphate buffer. A portion of the minced tissue was lysed in guanidium isothiocyanate, and total ribonucleic acid (RNA) was recovered by centrifugation through cesium chloride as previously described.[10] Poly(A) RNA was then selected by oligo (dT)-cellulose column chromatography.[11] Five micrograms of placental messenger RNA and 10 μg of endothelial cell total RNA from the human umbilical cord, taken as a positive control for endothelin gene expression, were then fractionated on 1.2% agarose gel with 6% formaldehyde and blotted onto synthetic membranes (Gene Screen Plus, New England Nuclear, Boston). Human preproendothelin probe was labeled by a random-primed labeling method with the use of (α-phosphorus 32)deoxycytidine triphosphate.[12] Membranes were hybridized with endothelin 1 complementary deoxyribonucleic acid labeled with phosphorus 32 and exposed to x-ray film for autoradiography.

To investigate the placental elution pattern of endothelin 1, 2, and 3 and Big endothelin 1, the tissue was frozen after collection, boiled for 10 minutes in 1 mol/L acetic acid to inactivate proteases, and then homogenized with an Ultra-Turrax (Janke & Kunkel GMBM & Co. KG, Staufen, Federal Republic of Germany) homogenizer in an ice bath for 60 seconds. After the addition of 0.5% Triton X-100 and 5000 counts/min of iodine 125–endothelin (Amersham International, Buckinghamshire, U.K.), the homogenate was shaken in an ice bath for 20 minutes, then centrifuged at 40,000 *g* for 30 minutes at 4° C. The supernatant was loaded into a Sep-pak C_{18} cartridge, which had been conditioned with 5 ml of methanol and 5 ml of water and preequilibrated with 5 ml of 1 mol/L acetic acid. After the sample was applied, the cartridge was washed with 5 ml of 1 mol/L acetic acid and the adsorbed material was eluted with 3 ml of acetonitrile/0.1% trifluoroacetic acid (65:35). Eluate was extracted with diethylether and the aqueous phase was subjected to reversed-phase high-performance liquid chromatography.

Extracted sample was chromatographed on a reversed-phase C_{18} column equilibrated with 0.1% trifluoroacetic acid. A gradient of acetonitrile was used at a flow rate of 1 ml/min. High-performance liquid chromatography fractions were collected and lyophilized, and the content of different endothelins was assayed in high-performance liquid chromatography fractions that coeluted with corresponding authentic standards. The protein content of placental tissue was determined by the method of Lowry et al.[13]

Blood was quickly mixed with ethylenediaminetetraacetate (2 mg/ml) and aprotinin (500 kallikrein inhibitor units per milliliter); plasma was separated by centrifugation at 1700 *g* for 15 minutes and immediately frozen at −20° C until assayed. Plasma samples (5 ml), spiked with 1000 counts/min of ^{125}I-endothelin, were applied to a Sep-pak C_{18} column, eluted as described for placental tissue, and lyophilized until assayed. Overnight urine samples were collected during 6 to 8 hours and frozen at −20° C until time of assay. Urine samples (5 ml) containing 1000 counts/min of ^{125}I-endothelin were run on a Sep-pak C_{18} disposable column that had been previously activated with 3 ml of 100% methanol, 3 ml of 10% methanol, 3 ml of 20% methanol, 3 ml of 85% methanol, and 4 ml of 10% methanol. The column was subsequently washed with 4 ml of 20% methanol, and elution was performed with 4 ml of 85% methanol. The elution fraction was lyophilized. The dried residue was stored at −20° C until assayed. The overall extraction recovery, as assessed by ^{125}I-endothelin, was 80% to 85% for placental tissue, 65% to 75% for plasma, and 70% to 85% for urine.

Extracted samples were resuspended in phosphate buffer (pH 7.2) containing 0.1% Triton X-100 and 0.3% albumin (radioimmunoassay buffer) immediately before the assay. Either standard peptides (endothelin 1, 2, or 3 or Big endothelin 1, Peptide Institute, Osaka) or unknown samples (100 μl) were incubated with 100 μl of the antiserum (for measurement of endothelin 1, 2, or 3: Peninsula Laboratories, Inc., Belmont, Calif.; for measurement of Big endothelin 1:Biomedica, Vienna) for 24 hours at 4° C. Then 12,000 counts/min of ^{125}I-endothelin or human ^{125}I-Big endothelin 1 (Amersham) (100 μl) was added and the incubation was prolonged for 24 hours at 4° C.

Separation of free from antibody-bound labeled peptides was achieved by addition of an immunoprecipitating mixture consisting of a sheep antirabbit immunoglobulin G and polyethylene glycol (500 μl) for 30 minutes at room temperature. Finally, 1 ml of phosphate buffer was added to stop the reaction and the immunoprecipitates were centrifuged at 1700 *g* for 20 minutes. Supernatants were removed by aspiration with a vacuum pump, and pellet radioactivity was detected by a γ-counter (Beckman, Irvine, Calif.). Results were

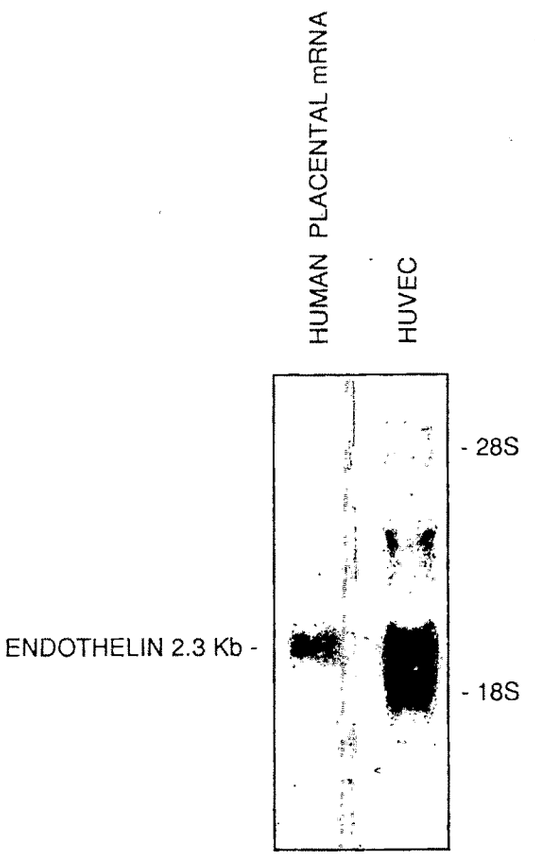


Fig. 1. Endothelin messenger RNA expression in placental tissue. RNA from umbilical vein endothelial cells *(HUVEC)* was used as positive control. Ribosomal RNAs (28S and 18S) were used as size markers.

expressed as picograms per milligram protein for placental tissue, picograms per milligram for plasma, and picograms per milligram creatinine for urine.

The minimum detectable concentration that could be measured was 0.4 pg per tube for endothelin 1, 2 pg per tube for endothelin 2, 0.8 pg per tube for endothelin 3, and 3.1 pg per tube for Big endothelin 1. Nonspecific binding did not exceed 2% of total radioactivity. For measurements of endothelin 1, 2, and 3 an anti–endothelin 1 antibody was used that cross reacts with endothelin 2 and 3, as follows: endothelin 1, 100%; endothelin 2, 46.9%; endothelin 3, 17%. Intraassay and interassay variability averaged 9% and 14% for all peptides measured. Validation of radioimmunoassay measurements was performed by comparison with different antisera and by recovery of cold endothelins added to placental tissue, plasma, and urine. Urine creatinine was measured by the alkaline picrate method.[14] All results are expressed by mean ± 1 SD. Data were analyzed by means of one-way analysis of variance with the use of Duncan's test for multiple comparison and the Student t test, as appropriate. All p values <0.05 were considered to indicate statistical significance.

Results

Normotensive pregnant subjects participating in this study ($n = 24$) ranged in age from 28 to 38 years. All women remained normotensive throughout pregnancy and had normal deliveries with a mean birth weight of 3.32 ± 0.35 kg. Placental tissue taken from these subjects constitutively expressed messenger RNA encoding preproendothelin. Fig. 1 shows a Northern blot demonstrating a single 2.3 kb preproendothelin messenger RNA expressed in placental tissue. This message was identical in size to that found in RNA obtained from human umbilical cord endothelial cells.

To investigate the placental endogenous content of endothelin 1, 2, and 3 and Big endothelin 1, placental tissue was extracted and subjected to high-performance liquid chromatography. The elution pattern of authentic standards separated by reversed-phase high-performance liquid chromatography is reported in Fig. 2, *a*. Three major peaks eluting from the high-performance liquid chromatography at the expected retention times of endothelin 3, Big endothelin 1, and endothelin 1 authentic standards were detected. Those values were on average 9.09 ± 0.01, 17.44 ± 3.50, and 12.20 ± 4.33 pg/mg of protein, respectively. A minor peak of immunoreactive material corresponding to endothelin 2 was observed (0.61 ± 0.14 pg/mg of protein) (Fig. 2, *b*). Endothelin plasma levels at delivery were numerically higher than those measured in normal nonpregnant subjects (0.35 ± 0.22 vs 0.24 ± 0.02 pg/ml), but the difference did not reach statistical significance. Urinary excretion of endothelin in the first trimester of pregnancy was numerically, although not significantly, increased (7.63 ± 4.31 pg/mg of creatinine) as compared with normal nonpregnant women (3.12 ± 2.65 pg/mg creatinine, Fig. 3). In the second and third trimesters urinary excretion of endothelin further increased, reaching statistically significant values (second trimester, 15.56 ± 4.00 pg/mg creatinine; third trimester, 19.57 ± 7.93 pg/mg creatinine; $p <$ 0.01) over those found in nonpregnant subjects and in the first trimester.

Comment

This study has documented that normal placental tissue expresses the endothelin gene and generates endothelin, a 21 amino acid peptide originally isolated from porcine aorta that induces hypertension in the rat when infused intravenously.[15]

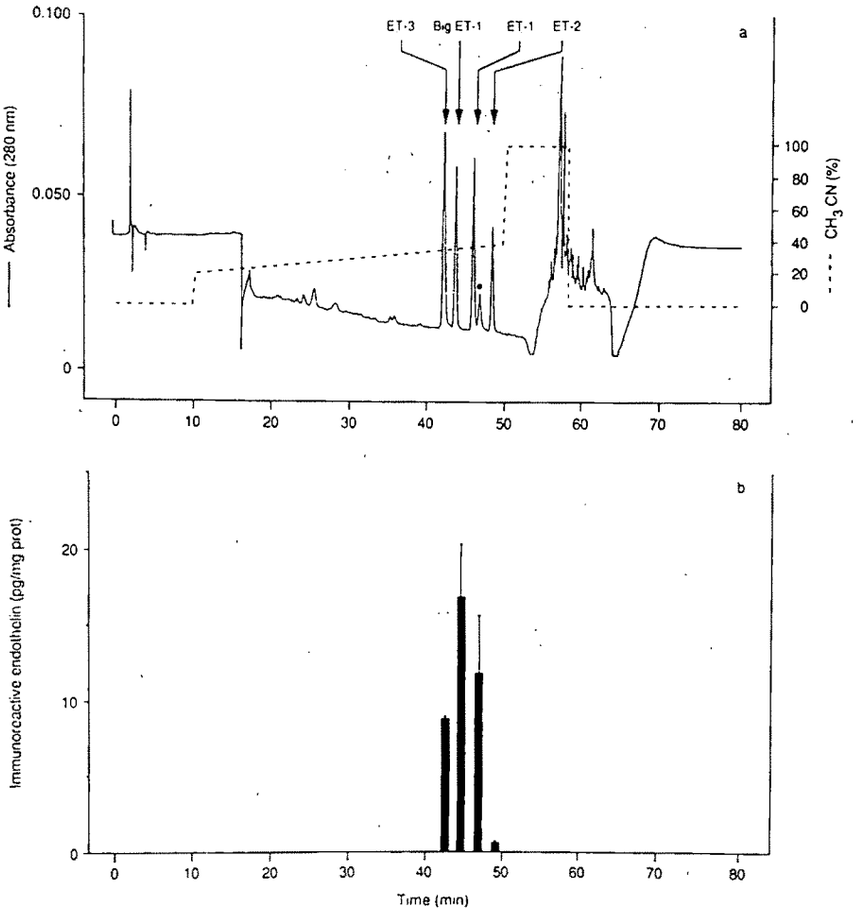


Fig. 2. a, Reversed-phase high-performance liquid chromatography elution profiles of the authentic standards of endothelin 3 *(ET-3)*, Big endothelin 1 *(Big ET-1)*, endothelin-1 *(ET-1)*, and endothelin-2 *(ET-2)*. *Asterisk*, Gradient peak. **b,** Immunoreactive profile of extract of human placenta. Each high-performance liquid chromatography fraction was concentrated by lyophilization and subjected to radioimmunoassay for *ET-3*, *Big ET-1*, *ET-1*, and *ET-2*.

Our results showed a numeric tendency for endothelin plasma levels to increase in pregnancy as compared with normal nonpregnant women, but the difference did not reach statistically significant values. These findings are in keeping with the majority of the data available, that circulating levels of endothelin are very low,[16, 17] suggesting that endothelin is not a circulating hormone but probably acts as a local regulator of blood supply to the various tissues.

We also wanted to investigate whether the renal synthesis of endothelin is modified in pregnancy. If so, it would have a major impact on the adaptation of renal circulation in pregnancy. Previous studies in experimental animals have documented that urinary excretion of endothelin may reflect renal synthesis of the hormone because labeled endothelin infused into systemic circulation of normal rat is excreted only in the

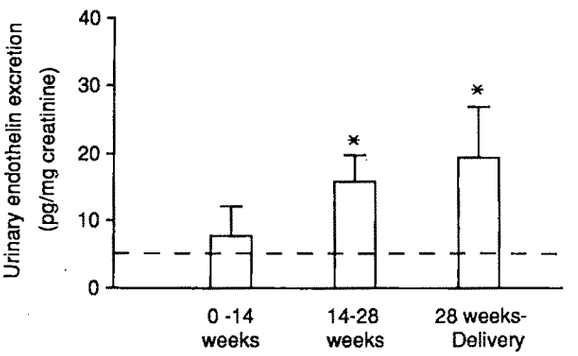


Fig. 3. Urinary excretion of endothelin in pregnant subjects throughout gestation. *Hatched line* represents upper 95% confidence limit of urinary endothelin excretion in normal nonpregnant women. *Asterisk*, $p < 0.01$ versus nonpregnant subjects and women in first trimester of pregnancy.

urine in negligible amounts. These results show that urinary excretion of endothelin progressively increased during normal pregnancy, reaching values statistically higher than in nonpregnant women at 14 to 28 weeks' gestation and increasing further thereafter.

Although there is no direct evidence that changes in endothelin renal synthesis are causally related to changes in renal hemodynamics, these results raise the possibility that renal endothelin acts as a local hormone to counteract the action of vasodilatory substances, such as prostacyclin and prostaglandin E_2 that markedly increase during normal pregnancy.[5, 6, 18] Endothelin potently stimulates the metabolism of arachidonic acid in various systems[19, 20]; therefore the possibility that changes in prostacyclin and prostaglandin E_2 are in fact mediated by endothelin can also be considered.

Further studies are needed to clarify the role of endothelin in the pathophysiologic characteristics of normal pregnancy and to establish whether the most common complications of pregnancy, such as hypertension and preeclampsia, are related to changes in endothelin biosynthesis or metabolism.

We are deeply indebted to Dr. Engelbert Hartter (2nd Department Internal Medicine, University of Vienna) for his cooperation, which allowed us to set up endothelin measurements in our Institute. We acknowledge Dr. Alessandro Rambaldi for helpful criticism and discussion. This study was possible because of the cooperation of Dr. Enio Cappato, Chief of the Division of Obstetrics and Gynecology, Ospedali Riuniti di Bergamo, and of Professor Attilio Gastaldi, Chief of the Obstetrics and Gynecology Clinic, University of Brescia.

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Combination antimicrobial therapy in the treatment of acute pelvic inflammatory disease

Daniel V. Landers, MD,[a] Pal Wolner-Hanssen, MD,[b] Jorma Paavonen, MD,[b] Edwin Thorpe, MD,[b] Nancy Kiviat, MD,[b] Marilyn Ohm-Smith, MS,[a] James R. Green, MD,[a] Julius Schachter, PhD,[a] King K. Holmes, MD, PhD,[b] David A. Eschenbach, MD,[b] and Richard L. Sweet, MD[a]

San Francisco, California, and Seattle, Washington

We compared the clinical and microbiologic efficacy of two broad-spectrum combination antimicrobial regimens in the treatment of 148 patients with acute pelvic inflammatory disease. Patients were randomized to inpatient treatment with either cefoxitin and doxycycline (n = 75) or clindamycin and tobramycin (n = 73). These antibiotics were administered intravenously for at least 4 days, and up to 48 hours beyond defervescence. Patients were discharged on a regimen of oral doxycycline or clindamycin in accordance with the intravenous regimen to complete a total duration of therapy of 2 weeks. *Neisseria gonorrhoeae* (53%) and *Chlamydia trachomatis* (31%) were the microorganisms that were isolated most frequently from the genital tract of enrolled patients. At follow-up, *N. gonorrhoeae* was isolated in two patients, and *C. trachomatis* was isolated in none. The overall initial favorable response rate to combination antimicrobial therapy was 98.5% (130/132) in patients with uncomplicated pelvic inflammatory disease and 81% (13/16) in patients with pelvic inflammatory disease that was complicated by tuboovarian abscess. A > 70% decrease in abdominal tenderness score occurred in 89% of 111 patients within 6 weeks of hospital discharge. There were no significant differences between antibiotic treatment groups in any response categories or in toxicity. During the initial hospitalization, five patients (three with tuboovarian abscess, one with a pyosalpinx, and one with intractable acute and chronic pelvic inflammatory disease) required surgical intervention. These results support the recommendation to use broad-spectrum combination antimicrobial therapy for the treatment of acute pelvic inflammatory disease. (Am J Obstet Gynecol 1991;164:849-58.)

Key words: Pelvic inflammatory disease, acute salpingitis, pelvic infections, sexually transmitted diseases

The variety of microorganisms that cause acute pelvic inflammatory disease has been well-documented in numerous reports.[1-5] Microorganisms most commonly isolated from the upper genital tract of patients with acute pelvic inflammatory disease include *Chlamydia trachomatis, Neisseria gonorrhoeae, Escherichia coli, Bacteroides bivius,* and peptostreptococci. Awareness of the complex microbiologic features of acute pelvic inflammatory disease resulted in concern that single-agent antimicrobial therapy may not be effective in treating all patients with the disease. Cephalosporins and aminopenicillins have been the most common single drugs given to patients hospitalized for pelvic inflammatory disease.[6] These drugs are effective against gonococci; however, an intravenous cephalosporin regimen failed to eradicate *C. trachomatis* in the majority of patients with acute chlamydial pelvic inflammatory disease.[7] Tetracycline, the drug of choice for *C. trachomatis,* is not effective in treating *N. gonorrhoeae.* It became obvious that no single drug was effective against the broad range of potential pathogens in pelvic inflammatory disease. This was supported by the observation of Thompson et al.[8] of clinical failure rates of 14% to 19% with single-agent outpatient therapy with ampicillin or tetracycline in the treatment of acute pelvic inflammatory disease. Thus, in 1982, 1985, and 1989, the Centers for Disease Control recommended combination antimicrobial therapy for pelvic inflammatory disease because of its polymicrobial nature.[9-11] While several combination regimens provided broad-spectrum activity in vitro and offered coverage for the wide range of expected pathogens, none had been adequately evaluated for clinical efficacy in the treatment of pelvic inflammatory disease. The recommendations of the

From the Departments of Obstetrics, Gynecology and Reproductive Sciences and Laboratory Medicine, University of California, San Francisco, San Francisco General Hospital,[a] and the Departments of Obstetrics and Gynecology, Pathology, and Medicine, University of Washington, Harborview Medical Center.[b]

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Reprint requests: Daniel V. Landers, MD, San Francisco General Hospital, Room 6D14, 1001 Potrero Ave., San Francisco, CA 94110.

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Centers for Disease Control included examples of two combination regimens for hospitalized women with pelvic inflammatory disease: (1) cefoxitin plus doxycycline and (2) clindamycin plus gentamicin. Recently, Walters et al.[12] reported equal efficacy on comparing clindamycin and gentamicin with cefoxitin and doxycycline. Soper and Despress[13] had similar findings when they compared clindamycin and amikacin with cefoxitin and doxycyline.

This study was undertaken as a prospective, randomized, unblinded clinical trial to compare the antimicrobial and clinical efficacy of the combination regimens listed in the Centers for Disease Control guidelines, for the therapy of hospitalized patients with acute pelvic inflammatory disease.

Material and methods

Population. Eligible participants in the study were women older than 16 who had been admitted to the San Francisco General Hospital or to the Harborview Medical Center in Seattle, Washington, with abdominal pain. These patients were thought to have acute pelvic inflammatory disease. Excluded were patients with an allergy to any of the four antibiotics involved in the trial, pregnant women, or those with a history of having received antibiotic therapy within 2 weeks before admission to the study. Also excluded were patients with a history of pelvic surgery, abortion, uterine curettage, or delivery within 6 weeks of admission. The study was reviewed and approved by the institutional review boards at both hospitals that were involved in patient recruitment. Written informed consent was obtained from all patients before enrollment.

Pretreatment evaluation. All patients enrolled had either laparoscopically confirmed salpingitis[14] or histologically confirmed plasma cell endometritis[15] or they met previously published criteria for the noninvasive diagnosis of salpingitis.[16] These criteria include direct abdominal tenderness, cervical motion tenderness, and adnexal tenderness, plus one or more of the following: temperature ≥38° C, peripheral blood leukocytosis white blood cell count >10,500 mm^3, purulent material or culdocentesis, evidence of pelvic abscess on ultrasonography or pelvic examination, evidence of gonococcal or chlamydial cervicitis (by positive monoclonal antibody test or by Gram's stain showing gram-negative intracellular diplococci), or mucopurulent cervicitis as previously defined.[17]

Pretreatment laboratory tests included a complete blood count; a platelet count; serum electrolyte, creatinine, and blood urea nitrogen tests; liver function tests; a serum pregnancy test; blood cultures; and urinalysis. Cervical cultures were obtained for *N. gonorrhoeae, C. trachomatis, Mycoplasma hominis,* and *Ureaplasma urealyticum.* Endometrial cultures were obtained for these agents and for other facultative and anaerobic microorganisms. Women who underwent laparoscopy also had specimens obtained from the fallopian tubes and cul-de-sac fluid for culture of these microorganisms. Ultrasonographic examination was performed on all patients with adnexal masses, a slow clinical response, or an indication of tuboovarian abscess. Complex adnexal masses >4 cm were judged to be consistent with tuboovarian abscess.

Microbiology. Endocervical specimens for *C. trachomatis* were obtained via either cotton-tipped swabs or calcium alginate swabs. These specimens were taken from lots that were pretested and found to be nontoxic for *C. trachomatis.* The swabs were placed in antibiotic containing minimal essential medium or phosphate sucrose buffer and refrigerated for transport to the laboratory within 16 hours after collection. To test for *N. gonorrhoeae,* specimens from the endocervix were obtained with a Dacron or cotton-tipped swab and plated directly onto modified Thayer-Martin agar plates. These specimens were held at 35° C in a candle jar until they were transported to the laboratory (within 6 hours of collection), where they were incubated at 35° C in 5% carbon dioxide. Endocervical *M. hominis* and *U. urealyticum* specimens were obtained via a Dacron swab, which was immediately inoculated to a A7B agar plate and U9B broth. These specimens were held in a candle jar at 35° C until they were transported to the laboratory (within 6 hours of collection), where the A7B agar plate was incubated at 35° C in 5% carbon dioxide. The U9B broth was incubated at 35° C in room air. Endometrial brush or biopsy samples were divided under sterile conditions. One part was processed for *C. trachomatis* isolation, and the other was processed for the isolation of facultative and anaerobic organisms.

Specimens for *C. trachomatis* isolation were inoculated onto monolayers of McCoy cells by centrifugation. The cycloheximide-treated cells were cultured for 65 hours, and then examined for inclusions by iodine staining or immunofluorescence. Specimens were processed for isolation of facultative organisms by inoculation onto the following media, which were incubated in 5% carbon dioxide at 35° C: trypticase soy agar with 5% sheep blood, chocolate agar, CNA agar with 5% human blood, modified Thayer-Martin agar, and CNA agar with 5% sheep blood. These plates were examined two to three times over a 3- to 4-day period. *N. gonorrhoeae* and facultative bacteria were identified by standard methods.[18] Specimens processed for anaerobic growth were inoculated into Brucella agar that was supplemented with 5% sheep blood, vitamin K, and hemin, CNA agar with 5% sheep blood, kanamycin-vancomycin laked blood (LKV) agar, and chopped meat carbohydrate broth. Specimens were incubated at 35° C in either a GasPak jar (BBL, Cockeysville, Md.) or an anaerobic

chamber (Coy Manufacturing, Ann Arbor). Anaerobic microorganisms were identified via gas-liquid chromatography and prereduced anaerobically sterilized media.[19] Identification of colonies of genital mycoplasmas was based on colony morphologic features. Dark brown colonies on A7B agar which indicated hydrolysis of urea, were identified as *U. urealyticum*. In the U9B broth the presence of *U. urealyticum* was confirmed by a color change from yellow to pink. Identification of *M. hominis* was based on the "fried egg" appearance of colonies on A7B agar and failure to hydrolyze urea.

Tenderness scoring. Abdominal and pelvic examinations were scored prospectively at Harborview Medical Center via a modification of the scoring system described by McCormack et al.[20] In this scoring system 0 is absent, 1 is minimal, 2 is moderate, and 3 is severe. A score of 0 to 3 was assigned for direct tenderness and for rebound tenderness of the right and left upper and lower abdominal quadrants (total possible score = 24). In addition, pain with cervical motion, uterine tenderness, and left and right adnexal tenderness were scored from 0 to 3 (total possible score = 12). Finally, right and left adnexal masses were scored (total possible score = 6). In the scoring of masses, 0 is no mass, 1 is mass <2 cm, 2 is mass 2 to 5 cm, and 3 is mass >5 cm. Thus the maximum possible score on the examination was 42. Whereas it was not part of the initial design, the same system was adapted for retrospective analysis of abdominal and pelvic examination of patients at San Francisco General Hospital.

Treatment regimens. Patients were randomly assigned to one of two treatment regimens. One group received cefoxitin, 2 gm intravenously every 6 hours, plus doxycycline, 100 mg intravenously every 12 hours. The other group received clindamycin, 600 mg intravenously every 6 hours, plus tobramycin 2.0 mg/kg intravenously for one dose, followed by 1.5 mg/kg intravenously every 8 hours. All intravenous antibiotics were continued for a minimum of 4 days and for at least 48 hours after disappearance of fever. Patients that were afebrile on admission were treated for a minimum of 4 days in the hospital for at least 24 hours beyond the adequate relief of pain and tenderness which allowed patients to return to a normal life-style without surgical intervention. After discharge from the hospital, either oral doxycycline, 100 mg twice daily, or oral clindamycin, 450 mg four times daily, was continued to complete a total of 14 days of treatment.

Follow-up evaluation. Patients were interviewed and examined both at hospital discharge and at 2 to 6 weeks after initial enrollment. Cervical cultures were repeated at each follow-up visit. An initial favorable response was defined as a patient who was afebrile and did not need surgical intervention.

Statistical analysis. Discrete variables were analyzed with a standard χ^2 test or two-tailed Fisher's exact when cell values were <5. Continuous variables were analyzed with the unpaired t test. All p values of <0.05 were considered statistically significant.

Results

Patient characteristics. Total enrollment in this study included 102 patients, 14 of which could not be evaluated. Reasons for exclusion were incorrect diagnosis that was discovered at laparoscopy or laparotomy (i.e., ectopic pregnancy, endometriosis, appendicitis) or refusal of the patient to remain hospitalized long enough to complete treatment.

There were 148 evaluable patients—89 at San Francisco General Hospital and 59 at Harborview Medical Center. The diagnosis of acute pelvic inflammatory disease was made by laparoscopy in 43 patients, by endometrial biopsy in another 13, and by clinical diagnosis in the remaining 92 patients. Seventy-five of the 148 patients enrolled were treated with cefoxitin in combination with doxycycline, and 73 were treated with clindamycin in combination with tobramycin.

The demographic and historic data on sexual habits, contraceptive methods, and sexually transmitted diseases are summarized in Table I. There were no significant differences between treatment groups for any of the variables listed. There were also no differences in any of the variables between the patient populations at San Francisco General Hospital and those at Harborview Medical Center, with the exception of previously identified chlamydial infection. This infection was reported significantly more often by Harborview Medical Center (10/46, 22%) than by San Francisco General Hospital (4/89, 5%) (population $p < 0.01$).

Temperature at admission was ≥38° C in 55 (38%) of 145 patients; the overall mean temperature at admission was 37.6 ± 0.7° C. The white blood cell count was increased (>10,500/mm^3) in 97 (67%) of 145 patients; the mean white blood cell count was 12,700/mm^3 ± 4500/mm^3. Erythrocyte sedimentation rate was elevated (>20 mm/hr) in 82 (57%) of 143 patients; the mean erythrocyte sedimentation rate was 32.5 ± 25.9 mm/hr. There were no differences in admission temperature, white blood cell count, or erythrocyte sedimentation rate between those with laparoscopic diagnosis and those without that diagnosis. There were no differences between study centers San Francisco General Hospital vs Harborview Medical Center in these values. There also were no differences in these values between treatment groups, with the exception of the mean erythrocyte sedimentation rate, which was higher in the clindamycin-tobramycin group than in the cefoxitin-doxycycline group (38.4 vs 27.7 mm/hr; $p < 0.03$), because of two very high erythrocyte sedimentation rate values. Median erythrocyte sedi-

Table I. Demographic and historical characteristics of enrolled patients

	Group 1. Cefoxitin-doxycycline (n = 75)	*Group 2. Clindamycin-tobramycin (n = 73)*	*Total (n = 148)*
Age (yr, mean ±SD)	23.3 ± 5.3	23.8 ± 6.0	23.5 ± 6.1
Race			
black	38 (50%)	36 (49%)	74 (50%)
white	29 (39%)	32 (44%)	61 (41%)
Hispanic	5 (7%)	4 (5%)	9 (6%)
other	3 (4%)	1 (1%)	4 (3%)
Gravidity			
0	14 (19%)	23 (32%)	37 (25%)
≥1	61 (81%)	50 (68%)	111 (75%)
Parity			
0	40 (53%)	42 (58%)	82 (55%)
≥1	35 (47%)	31 (42%)	66 (45%)
Birth control method	n = 74	n = 72	n = 146
Rhythm or none	46 (62%)	56 (78%)	102 (70%)
Oral contraceptives	11 (15%)	6 (8%)	17 (12%)
Intrauterine contraceptive device	4 (5%)	2 (3%)	6 (4%)
Barrier	9 (12%)	7 (9%)	16 (11%)
Tubal ligation	4 (5%)	1 (1%)	5 (3%)
Prior gonorrhea	34/74 (46%)	27/73 (37%)	61/147 (41%)
Prior chlamydia	11/71 (15%)	7/71 (10%)	18/142 (13%)
Prior pelvic inflammatory disease	23/75 (31%)	25/71 (35%)	48/146 (33%)
No. of partners in last 30 days	n = 72	n = 73	n = 145
0	5 (7%)	4 (5%)	9 (6%)
1	41 (57%)	47 (64%)	88 (61%)
≥2	26 (36%)	22 (30%)	48 (33%)
Partners during lifetime	n = 67	n = 64	n = 131
1	2 (3%)	4 (6%)	6 (5%)
2-10	39 (58%)	38 (59%)	77 (59%)
11-50	19 (28%)	11 (17%)	30 (23%)
>50	7 (10%)	11 (17%)	18 (14%)
Days since last intercourse	n = 67	n = 63	n = 130
1	2 (3%)	4 (6%)	6 (5%)
7	39 (58%)	38 (60%)	77 (59%)
8-30	19 (28%)	11 (17%)	30 (23%)
>30	7 (10%)	10 (16%)	17 (13%)

mentation rate values were not significantly different (30 vs 22 mm/hr).

Clinical response. The criteria for a satisfactory initial clinical response which were defined as an improvement of admitting signs and symptoms, included abdominal-pelvic pain, fever, and pelvic tenderness. An initial satisfactory clinical response occurred for 143 (97%) of 148 patients. The diagnosis of tuboovarian abscess was made by clinical examination with confirmation by ultrasonography or by either laparoscopy or laparotomy. A satisfactory rate of response was documented for 130 (98.5%) of 132 patients who had uncomplicated pelvic inflammatory disease and for 13 (81%) of 16 patients with tuboovarian abscess (NS). One of these patients underwent a primary laparoscopic drainage of the abscess at the time antibiotic therapy was initiated; therefore a satisfactory response was documented for 12 (80%) of 15 patients with abscesses who had received antibiotics alone. There were no differences in the response rate between antibiotic treatment groups for patients with either acute uncomplicated pelvic inflammatory disease or acute pelvic inflammatory disease with tuboovarian abscess (Table II).

Five patients needed surgery during the initial hospitalization. Two of these patients from the cefoxitin-doxycycline group had tuboovarian abscess. Three patients were from the clindamycin-tobramycin group-one had a tuboovarian abscess, one had pyosalpinx, and one had chronic persistent pelvic inflammatory disease.

Five patients returned with recurrent signs and symptoms of pelvic inflammatory disease within 6 weeks of discharge; three had been treated with cefoxitin-doxycycline, and two had received treatment with clindamycin-tobramycin. It was not possible to determine if these patients were reinfected or if they experienced recurrence or a recrudescence of the initial infection. None of the five had a positive culture for *C. trachomatis* or *N. gonorrhoeae* at the time of the recurrence of signs and symptoms of pelvic inflammatory disease.

The mean tenderness score on admission was 17.9 ± 5.9. No difference was found in initial mean tenderness

Table II. Favorable initial clinical response to indicated therapy

Diagnosis	*Cefoxitin-doxycycline*	*Clindamycin-tobramycin*	*Total*
Acute pelvic inflammatory disease	66/66 (100%)	64/66 (97%)	130/132 (98.5%)
Tuboovarian abscess	7/9 (78%)	6/7* (86%)	13/16 (81%)
TOTAL	73/75 (97%)	70/73 (96%)	143/148 (97%)

There were no significant differences in the clinical responses between treatment groups.
*One patient in this group underwent laparoscopic drainage of an abscess as a part of the initial therapy.

scores between treatment groups. No significant differences were found in tenderness scores, and whether the scores were obtained prospectively made no difference. Follow-up tenderness scores were available on the day of discharge and 2 to 6 weeks after enrollment in the studies. A >70% decrease in tenderness score was noted in 99 (89%) of 111 patients 2 to 6 weeks after treatment. Only five patients showed <20% improvement in tenderness score, and four of these underwent surgical intervention. There was no significant difference between treatment groups with respect to improvement in tenderness scores.

Of the 57 patients prospectively scored for tenderness at Harborview Medical Center, 38 (67%) of 57 had >70% reduction in tenderness at the time of discharge (Table III). A ≥70% reduction in tenderness was achieved for 12 (75%) of 16 patients, who initially had <70% reduction in tenderness at discharge and were reevaluated 2 to 4 weeks after the conclusion of therapy. However, of 31 patients considered cured at discharge (>70% reduction in tenderness score at discharge) and who were later reevaluated, increased tenderness developed in six and they were then considered only improved (<70% and >20% reduction in tenderness score) 2 to 4 weeks after therapy. There were no differences in the follow-up tenderness scores between antibiotic treatment groups.

Microbiologic features. The microorganisms most frequently isolated from study patients were *C. trachomatis* and *N. gonorrhoeae*. Of the 136 patients from whom both endocervical and endometrial cultures were obtained, *C. trachomatis* was isolated from 42 (31%) and *N. gonorrhoeae* from 72 (53%). At least one of these two types of sexually transmitted microorganisms was isolated from 92 (68%) of the study patients, and both types of microorganisms were isolated from 22 (16%) of 136 patients. *C. trachomatis* was recovered from the endometrium in 24 (69%) of 35 patients with documented endocervical *C. trachomatis* infection. An additional three patients had positive endocervical cultures, but no endometrial cultures were done. Conversely, this microorganism was isolated from the endocervix in 27 (90%) of 30 patients who had positive endometrial cultures for *C. trachomatis*. *N. gonorrhoeae* was recovered from the endometrium of 56 (79%) of the 71 patients with positive results for *N. gonorrhoeae* on endocervical cultures. Another eight patients with endocervical *N. gonorrhoeae* did not have endometrial culture results. Only one of the 57 patients with positive endometrial cultures for *N. gonorrhoeae* had a negative endocervical culture for this organism. Two additional positive endometrial cultures for *N. gonorrhoeae* were obtained in patients without endocervical *N. gonorrhoeae* cultures.

C. trachomatis was isolated from the fallopian tubes of 5 (12%) of 43 patients from whom pretreatment tubal cultures were obtained at laparoscopy and from two of the nine patients with *C. trachomatis* in the endometrium. Four of 17 patients with endocervical *C. trachomatis* infection who underwent pretreatment tubal cultures had documented tubal *C. trachomatis* infection. One patient with tubal *C. trachomatis* infection had a negative endocervical culture for this organism. *N. gonorrhoeae* was isolated from the fallopian tubes in 16 (37%) of 43 patients, all 16 of whom also had *N. gonorrhoeae* in endometrial and endocervical cultures. There was no significant correlation between treatment failure and the organisms isolated from either the fallopian tubes or the endometrium.

Results of cultures for *C. trachomatis* and *N. gonorrhoeae*, with respect to both treatment centers and antibiotic treatment groups, are summarized in Table IV. Both *C. trachomatis* and *N. gonorrhoeae* were isolated from the endocervix significantly more often from patients enrolled at Harborview Medical Center than from patients enrolled at San Francisco General Hospital. Patients enrolled at Harborview Medical Center were also more likely than patients at San Francisco General Hospital to have *N. gonorrhoeae* isolated from the endometrium ($p < 0.01$), but there was no significant difference between treatment centers in the rate of *C. trachomatis* isolation from the endometrium. There were no differences between antibiotic treatment groups in isolation rates of either *C. trachomatis* or *N. gonorrhoeae* from the endocervix or endometrium.

Numerous facultative and anaerobic microorganisms were isolated from endometrial specimens, and such microorganisms were isolated less often from fallopian tube specimens. The mean number of these microorganisms recovered from endometrium was 4.5. The

Table III. Reduction in prospective tenderness scores with antibiotic treatment alone

	>70% Reduction at discharge	*<70% and >20% Reduction at discharge; ≥70% reduction at 2-4 wk*	*Total with ≥70% reduction*
Cefoxitin-doxycycline	19/28	3/6*	22/28† (79%)
Clindamycin-tobramycin	19/29	9/10*	28/29 (97%)
TOTAL	38/57 (67%)	12/16 (75%)	50/57 (88%)

*Cases with <70% reduction in tenderness score at discharge who returned for the 2 to 4-week follow-up and who had ≥70% reduction in tenderness score at 2 to 4 weeks.

†In 28 patients treated with cefoxitin-doxycycline, reduction in tenderness score was reached in at least 22. Three patients who had no improvement at the time of discharge were not seen at follow-up.

Table IV. Comparisons of chlamydial and gonococcal isolates between treatment centers and between study groups

	San Francisco General Hospital	*Harborview Medical Center*	*p Value*	*Cefoxitin-doxycycline*	*Clindamycin-tobramycin*	*p Value*
Endocervix						
C. trachomatis	14/89 (16%)	24/59 (41%)	0.001	17/76 (22%)	21/73 (29%)	0.5
N. gonorrhoeae	40/87 (46%)	38/57 (67%)	0.02	39/74 (53%)	39/72 (54%)	1.0
Endometrial						
C. trachomatis	19/89 (21%)	11/49 (22%)	0.9	10/70 (14%)	20/68 (29%)	0.05
N. gonorrhoeae	31/89 (35%)	28/47 (60%)	<0.01	33/68 (49%)	26/68 (38%)	0.3

most common pathogens isolated from the endometrium (other than *C. trachomatis* or *N. gonorrhoeae*) were *Bacteroides* species ($n = 75$) (*B. bivius* ($n = 41$), *B. capillosus* ($n = 22$), *B. disiens* ($n = 12$), *Peptostreptococcus anaerobius* ($n = 47$), *P. asaccharolyticus* ($n = 46$), *P. tetradius* ($n = 20$), *P. prevotii* ($n = 16$), *Gardnerella vaginalis* ($n = 61$), coagulase-negative staphylococci ($n = 39$), nonhemolytic streptococci ($n = 27$) and α-hemolytic streptococci ($n = 19$).

Follow-up cultures for *C. trachomatis* and/or *N. gonorrhoeae* that were obtained 2 to 4 weeks after the completion of therapy were negative from 86 of 88 patients who initially had these organisms. One patient treated with cefoxitin-doxycycline was clinically and microbiologically cured at the 2-week follow-up visit but had positive culture for *N. gonorrhoeae* at the 6-week visit after sexual contact with a new sex partner. The other patient showed clinical improvement after intravenous treatment with cefoxitin-doxycycline, but culture was positive for *N. gonorrhoeae* and she had pain and tenderness at the 2-week follow-up visit. The first case probably represents reinfection; however, the second case represents possible persistent infection. Of patients with pretreatment *C. trachomatis* infection, none of 19 in the cefoxitin-doxycycline group and none of 20 in the clindamycin-tobramycin group had *C. trachomatis* that was isolated after therapy had concluded.

Mycoplasma hominis was isolated from 22 (36%) of 62 pretreatment endometrial cultures and from 12 (19%) of 63 posttreatment cultures. There was no difference between treatment groups in the proportion with pretreatment *M. hominis*; however, posttreatment isolation of *M. hominis* occurred in 12 of 34 patients in the cefoxitin-doxycycline group and in none of 29 patients in the clindamycin-tobramycin group ($p < 0.01$).

Adverse effects. No serious adverse effects from treatment were encountered by patients during this study. Gastrointestinal complaints occurred in several patients, but they were generally mild and did not lead to the discontinuation of the regimen in any cases. Nausea or vomiting occurred in 21 (21%) and diarrhea in 19 (19%) of 101 patients, but no significant differences in these symptoms occurred between antibiotic treatment groups. In the majority of these cases, the nausea was mild, without vomiting. In no case was it necessary to discontinue antibiotic treatment because of persistent symptoms. In two patients in the cefoxitin-doxycycline group and in one in the clindamycin-tobramycin group a mild rash developed. Blood urea nitrogen and serum creatinine were tested before and after completion of therapy in 47 patients, and all test results were within the normal range both before and after therapy. There were no differences between treatment groups in the increase of blood urea nitrogen or creatinine within the normal range. Serum glutamic oxaloacetic transaminase levels were tested before and after therapy in 34 patients. Abnormal serum glutamic oxaloacetic transaminase elevations occurred in eight patients, five of

whom had abnormal serum glutamic oxaloacetic transaminase values before treatment; no difference occurred in this value between antibiotic treatment groups.

Comment

N. gonorrhoeae has been linked with acute pelvic inflammatory disease since the early 1900's. Although the recovery of *N. gonorrhoeae* varies widely depending on the population, the microorganism is usually isolated from 30% to 50% of patients with pelvic inflammatory disease in the United States.[1, 2, 4, 20, 21] *C. trachomatis* has only recently been recognized to cause pelvic inflammatory disease; usually *C. trachomatis* is isolated in 25% to 50% of women with pelvic inflammatory disease.[3, 7, 22-28] *C. trachomatis* has been particularly associated with long-term sequelae of pelvic inflammatory disease, such as infertility and ectopic pregnancy.[28, 29] In this report *N. gonorrhoeae* was isolated from one half of the patients with acute pelvic inflammatory disease, *C. trachomatis* was isolated from one third of these patients, and either *N. gonorrhoeae* or *C. trachomatis* was isolated from more than two-thirds of these patients. Usually it is not possible to determine which of these bacteria are present at the time of antibiotic initiation. Because these microorganisms are isolated frequently, antibiotic regimens used for the treatment of pelvic inflammatory disease should be effective against both *N. gonorrhoeae* and *C. trachomatis*.

This report represents a multicenter direct comparison of the first two combination antimicrobial regimens recommended by the Centers for Disease Control for the treatment of hospitalized patients with pelvic inflammatory disease.[9-11] These regimens were recommended because they treat *N. gonorrhoeae*, and it was anticipated that they would adequately treat *C. trachomatis*. In fact, both cefoxitin-doxycycline and clindamycin-tobramicin appeared to effectively eradicate *C. trachomatis* from our patients. Doxycycline is more active than clindamycin against *C. trachomatis* in vitro, but the multidose administration of clindamycin at the dose used in this report also appears to be effective. Additionally, these regimens have good activity against the facultative and anaerobic microorganisms that are associated with pelvic inflammatory disease, although it was not possible to obtain posttherapy tubal cultures from our patients.

The antimicrobial regimens used in this study are not those traditionally chosen to treat patients with acute pelvic inflammatory disease. The majority of patients with pelvic inflammatory disease have continued to be treated as outpatients with a single antibiotic,[6] and many of the commonly selected oral antibiotics do not effectively inhibit *C. trachomatis*. In addition, the most frequently used inpatient therapies also have consisted of single antibiotics, the most common of which are cephalosporins and aminopenicillins.[6] While these single-agent regimens would be expected to eliminate *N. gonorrhoeae*, cephalosporins are particularly ineffective against *C. trachomatis*. In the best available example, *C. trachomatis* was reisolated from 12 of 13 patients with pelvic inflammatory disease who were treated only with cephalosporins.[7] Tetracyline has good activity against *C. trachomatis*, but it is disturbing that the use of tetracycline for hospitalized patients has actually decreased from 30% in 1966 to 20% in 1983,[6] during a time in which the importance of *C. trachomatis* in pelvic inflammatory disease was increasingly recognized.[5, 23, 25, 26]

The microbiologic makeup of acute pelvic inflammatory disease consists of a wide variety of facultative and anaerobic microorganisms, including *N. gonorrhoeae* and *C. trachomatis*. In this study we recovered *N. gonorrhoeae* from 53% of the patients evaluated and *C. trachomatis* from 31%. The recovery rates of these microorganisms were less (43% and 22%, respectively) from the endometrium. Thirty-seven percent of the 43 patients who had fallopian tube cultures had *N. gonorrhoeae*, and 12% of these patients had *C. trachomatis*.

The organisms were similar to those recovered from the upper genital tract of women with acute pelvic inflammatory disease in studies from Scandinavia and the United States.[1-4] *C. trachomatis* was the most common individual organism recovered from the upper genital tract of women with pelvic inflammatory disease in Scandinavia; however, Heinonen et al.[30] and Paavonen et al.[31] noted that anaerobic and/or facultative bacteria were recovered from 68% (17/25) and 69% (24/35) of their patients. In the United States, Wasserheit et al.[26] and Sweet[28] have reported recovery of anaerobic and/or facultative bacteria from the upper genital tract in 44% (11/23) and 69% (115/188) of patients with acute pelvic inflammatory disease. Interestingly, many of these anaerobic and facultative bacteria are the microorganisms that are associated with bacterial vaginosis.[32] Eschenbach[33] initially suggested that bacterial vaginosis might be a precursor for the development of pelvic inflammatory disease as the result of anaerobic and/or facultative bacteria. Recently, Paavonen et al.[31] demonstrated that such an association between bacterial vaginosis and pelvic inflammatory disease does exist and reported that bacterial vaginosis was present in 9 (29%) of 31 women with acute pelvic inflammatory disease, whereas it was not present in any of the 14 control patients. Thus regimens used to treat endometritis and salpingitis also should adequately treat these microorganisms.

The endometrial isolation of *M. hominis* was not appreciably reduced by the cefoxitin-doxycycline regimen, although *M. hominis* was eliminated by

clindamycin-tobramycin. The failure of doxycycline to eradicate *M. hominis* is probably related to the increasing prevalence of plasmid-mediated, tetracycline-resistant *M. hominis* strains.[34] If the goal of treatment is to render the upper genital tract sterile, then with respect to *M. hominis*, the cefoxitin-doxycycline regimen is not effective. However, women with *M. hominis* did not have symptoms, and ultimately the importance of *M. hominis* in pelvic inflammatory disease is unclear.[28] Although *M. hominis* can be recovered from the fallopian tubes of a small number of women with pelvic inflammatory disease,[1, 2, 35] the microorganism does not appear to ascend intracanalicularly from the cervix to infect the human fallopian tube.[34] Moreover, in vitro studies have failed to demonstrate that *M. hominis* produces adverse tissue destruction of the tubal epithelium,[36] in contradistinction to the tubal epithelium destruction demonstrated in tubal explants with *N. gonorrhoeae*[37] and in animal models for *C. trachomatis*[38] and *B. fragilis*.[39]

In previous studies up to one third of patients with a clinical diagnosis of pelvic inflammatory disease did not have salpingitis confirmed by laparoscopy.[14, 24-26] Inclusion of patients with no disease or with other conditions in antibiotic trials may alter the apparent clinical response. In this study patients had either laparoscopy, endometrial biopsy, or a constellation of clinical findings that reduced the number of those with no disease or with other conditions to a minimum. The clinical response of patients given either treatment regimen was judged to be satisfactory in 98% of patients with uncomplicated acute pelvic inflammatory disease. Too few patients were studied to detect a true difference (β-error) between drug regimens, if in fact a difference exists. In trials with other antibiotics, high degrees of efficacy judged by clinical parameters also have been observed,[5, 8, 21, 26] but patients with *C. trachomatis* were not analyzed as a separate group. In this trial the initial clinical response was high, not only for patients with *N. gonorrhoeae* but also for those with *C. trachomatis*. The clinical response appears to be better for patients with uncomplicated pelvic inflammatory disease than for patients with tuboovarian abscesses, but >80% of patients with tuboovarian abscesses showed a favorable clinical response to the antibiotic regimens used in this study.

The tenderness score was used in an attempt to evaluate more subtle response differences than was possible with an overall clinical response. Posttreatment tenderness scores were similar for patients treated with either drug regimen. However, reduction of the tenderness occurred slowly, and only two thirds of the patients had >70% reduction of tenderness at the time of hospital discharge. Some of these patients had a subsequent increase in tenderness. The tenderness score was used in an attempt to refine the clinical response; however, this assessment is still a crude measure of the response of infection to antibiotic therapy. In addition, the tenderness score has not been correlated with direct laparoscopic observations of the pelvis to determine whether persistent tenderness indicates a poor response, as indicated by persistent pelvic infection or by anatomic pelvic abnormalities, or whether persistent tenderness in a subset of patients is unassociated with objective evidence of disease. We support the attempts to develop better criteria to assess outcome.[40] Considerably more study of short-term (early) and long-term (late) outcome is needed. Short-term effects of therapy on the fallopian tubes can be evaluated directly by laparoscopy or by hysterosalpingography that is performed several months after therapy has concluded, although the approach is expensive. Long-term outcomes can be evaluated by assessing persistent pain, infertility rates, ectopic pregnancy rates, and need for surgical intervention of chronic pelvic pain.

Cost comparison between the two regimens includes not only the cost of the antibiotics but also charges for mixing and preparation and for administering each dose. We noted dramatic differences in the costs of these various services between the two hospitals. While the drug costs were higher in the cefoxitin-doxycycline group, higher total hospital costs were noted in the clindamycin-tobramycin group in spite of a similar number of hospitalization days between the groups. Thus meaningful cost comparisons between the two regimens is not possible. The current recommendation of the Centers for Disease Control to use gentamicin and dosing of clindamycin every 8 hours is probably designed to reduce the cost of this regimen.

In summary, the regimens recommended by the Centers for Disease Control for the treatment of hospitalized patients with pelvic inflammatory disease (either cefoxitin in combination with doxycycline or clindamycin in combination with an aminoglycoside) were highly and equally effective. No serious or significant side effects or toxicity was associated with either regimen. However, there are both short-term and long-term assessments of the treatment of acute pelvic inflammatory disease. The short-term assessment is based on immediate clinical response to treatment, reduction of hospitalization time, and prevention of relapse. On the other hand, the long-term goal, which may be even more important, is to preserve fallopian tube function, that is, to prevent tubal factor infertility and/or ectopic pregnancy.

Westrom,[41] in a cohort study in Sweden, demonstrated that the overall infertility rate was 21% after one or more (laparoscopically) documented episodes of acute pelvic inflammatory disease. He noted a direct linear relationship between the number of episodes of

infection and the infertility rate, which ranged from 11% for a single episode to >50% in women with three or more episodes. In addition, he reported a sixfold increase in tubal ectopic pregnancy among women with acute pelvic inflammatory disease. No such large-scale study to assess the long-term sequelae of acute pelvic inflammatory disease has been reported in the United States.[42] However, Brunham[43] in Canada recently described poor fertility prognosis in 7 of 23 laparoscopically confirmed pelvic inflammatory disease cases in which pregnancy attempted. All patients with adverse reproductive outcomes had either *C. trachomatis* infection or abscess formation, which is predominantly associated with anaerobic bacteria.

Our results support the use of broad-spectrum combination therapy for the treatment of acute pelvic inflammatory disease, although improved methods of measuring both short-term and long-term response to therapy are needed. Future studies should determine the long-term sequelae that develop after various inpatient versus outpatient treatment regimens. Patients must undergo long-term follow-up to determine their ultimate response to therapy and the effect of various treatment regimens on future infertility, ectopic pregnancy, and recurrence of pelvic inflammatory disease.

Finally, this study clearly demonstrates the effectiveness of high-dose clindamycin for the treatment of *C. trachomatis* infection in the setting of acute pelvic inflammatory disease. In none of the 20 cases with chlamydial infection in the clindamycin group was the organism recovered ≥3 weeks after treatment. This finding, plus the previous efficacy of clindamycin for treatment of chlamydial pelvic inflammatory disease as noted by Wasserheit et al.[26] raises serious questions about the most recent recommendations from the Centers for Disease Control for the treatment of hospitalized patients with acute pelvic inflammatory disease. In these recommendations follow-up treatment with doxycycline is suggested for patients initially treated with the clindamycin and gentamicin regimen.[11] It seems clear that clindamycin in high doses is adequate therapy for *C. trachomatis* infection in women.

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Meconium-stained amniotic fluid: A risk factor for microbial invasion of the amniotic cavity

Roberto Romero, MD, Shuichi Hanaoka, MD, Moshe Mazor, MD, Apostolos P. Athanassiadis, MD, Robert Callahan, BS, Yu Chiung Hsu, MD, Cecilia Avila, MD, Jose Nores, MD, and Carlos Jimenez, MD

New Haven, Connecticut

The purpose of this study was to determine whether meconium-stained amniotic fluid is a marker for microbial invasion of the amniotic cavity. Amniocentesis was performed on 707 patients presenting with preterm labor and intact membranes. Meconium-stained amniotic fluid was present in 4.2% (30/707) of patients with preterm labor. The prevalence of positive amniotic fluid cultures was significantly higher in women with meconium-stained amniotic fluid than in women with clear fluid (33% [10/30] vs 11% [75/677]; $p = 0.001$; odds ratio = 4.01; 95% confidence interval = 1.6 to 9.4). Patients with meconium-stained amniotic fluid were also more likely to have failed tocolysis and delivered a preterm neonate more frequently than patients with clear fluid (83% [25/30] vs 38% (258/677); $p = 0.0001$; odds ratio = 8.1; 95% confidence interval = 2.9 to 24.4). We conclude that meconium-stained amniotic fluid is a risk factor for microbial invasion of the amniotic cavity and preterm delivery in women with preterm labor and intact membranes. (Am J Obstet Gynecol 1991;164:859-62.)

Key words: Preterm labor, meconium, amniotic fluid, parturition, chorioamnionitis, intraamniotic infection, tocolysis, prematurity, discolored amniotic fluid, *Listeria monocytogenes*

Meconium-stained amniotic fluid is traditionally considered a sign of fetal hypoxia (reviewed in detail in reference 1). However, fetal acidosis is present in only a small fraction of cases with meconium-stained amniotic fluid, and the cause of and mechanisms responsible for meconium passage in the remainder of cases remain undetermined.[1] Intraamniotic infection with *Listeria monocytogenes* is associated with meconium-stained amniotic fluid.[2] Although the mechanism underlying this phenomenon is unknown, ingestion of infected amniotic fluid may lead to fetal enteritis and passage of meconium. Therefore the possibility must be considered that meconium-stained amniotic fluid may be a marker for microbial invasion of the amniotic cavity.

To address this question, we constructed a study of women presenting with preterm labor and intact membranes. This population was chosen because amniocentesis is routinely performed to screen for microbial invasion of the amniotic cavity.

From the Department of Obstetrics and Gynecology, Yale University School of Medicine.
Supported by a grant from the Walter Scott Foundation for Medical Research. Dr. Romero is the recipient of a Physician Scientist Award from the National Institute of Child Health and Human Development.
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Reprint requests: Roberto Romero, MD, Yale University School of Medicine, Department of Obstetrics and Gynecology, 333 Cedar St., P.O. Box 3333, New Haven, CT 06510.
6/1/26107

Material and methods

Patient population. Patients admitted to Yale–New Haven Hospital with the diagnosis of preterm labor and intact membranes from April 1, 1985, to March 31, 1989, underwent amniocentesis for evaluation of the microbiologic status of the amniotic fluid. Immediately after amniocentesis, the color of amniotic fluid was recorded by the operator.

Preterm labor was defined as the presence of regular uterine contractions with a frequency of at least two every 10 minutes associated with cervical change. Nitrazine, pooling, and ferning tests were performed in every case during a sterile speculum examination to exclude leakage of amniotic fluid into the vagina. Ritodrine, terbutaline, or magnesium sulfate was administered intravenously for tocolysis. Failure of tocolysis was diagnosed when cervical dilatation progressed beyond 3 cm and/or delivery occurred.

Microbiologic culture technique. Amniotic fluid was transported to the laboratory in a capped plastic syringe immediately after collection. These conditions precluded air contact with the specimen. Plating occurred

Table I. Microbial isolates from patients with meconium-stained amniotic fluid and positive cultures

Microorganism	*Incidence*	*Colony count (CFU/ml)*
Mixed anaerobic bacteria	4	10^2, 10^5, 10^2, 10^4
Ureaplasma urealyticum	3	NA
Listeria monocytogenes	2	$>10^5$, NA
Mycoplasma hominis	1	NA
Streptococcus viridans	1	$>10^5$
Peptococcus asaccharolyticus	1	1600
Bacteroides fragilis	1	$>10^5$

NA, Not available.

within 30 minutes of collection in most cases. Amniotic fluid was cultured for aerobic and anaerobic bacteria, as well as for *Mycoplasma sp.* (*Mycoplasma hominis* and *Ureaplasma urealyticum*), using methods previously described.[3] An intraamniotic infection was defined as the presence of a positive amniotic fluid culture for microorganisms.

Statistical analysis. Analysis of the data was conducted with a χ^2 test (True Epistat, Epistat Services, Richardson, Tex.).

Results

During the study period, there were 707 patients with the diagnosis of preterm labor and intact membranes whose amniocentesis reports contained information about the color of the amniotic fluid. Meconium-stained amniotic fluid was detected in 4.2% of cases (30/707). The prevalence of positive amniotic fluid cultures for microorganisms was higher in women with meconium-stained fluid than in women with clear amniotic fluid (33% [10/30] vs 11% [75/677]; $p = 0.001$; odds ratio = 4.01; 95% confidence interval = 1.6 to 9.4).

Table I displays the microbiologic findings in the 10 cases of patients with meconium-stained amniotic fluid. It is noteworthy that there were two cases with *Listeria monocytogenes* intraamniotic infection. The most prevalent microorganism was *Ureaplasma urealyticum* ($n = 3$).

There were no differences in gestational age on admission, interval to delivery, and birth weight between women with meconium and positive cultures and those with meconium and negative cultures (Table II).

Women with meconium-stained amniotic fluid were more likely to have failure of tocolysis and be delivered of a preterm neonate than women without meconium (83% [25/30] vs 38% [258/677]; $p = 0.0001$; odds ratio = 8.1; 95% confidence interval = 2.9 to 24.4).

Comment

Pathologic studies have documented an association between meconium-stained amniotic fluid and the presence of inflammatory lesions of the placenta and membranes.[4] These lesions were originally attributed to the irritant effect of meconium. Subsequent studies demonstrated that injection of sterile meconium into animals failed to induce an inflammatory tissue response.[5] An alternative explanation for these pathologic findings is that meconium is passed when there is microbial invasion of the amniotic cavity. This tenet is consistent with previous observations in which infants born to mothers with meconium-stained fluid had a higher incidence of perinatal infections.[6] Our data support an association between meconium-stained amniotic fluid and intraamniotic infection in the context of preterm labor.

Three general possibilities might be considered to explain our findings: (1) Swallowed bacteria may induce meconium passage; (2) meconium may impair the antibacterial properties of amniotic fluid and promote bacterial growth; (3) an intrauterine ascending infection may lead to decidual bleeding and the appearance of meconium-like amniotic fluid.[7]

There is a striking association between intraamniotic infection with *Listeria monocytogenes* and meconium-stained amniotic fluid.[2] The mechanism postulated to explain this phenomenon is that ingestion of infected amniotic fluid leads to a fetal enteritis and subsequent meconium passage. It is unclear why some intraamniotic infections are associated with meconium-stained fluid whereas others are not. The type and virulence of the organism, inoculum size, and gestational age of the fetus may be important factors. For example, *Listeria monocytogenes* is a particularly virulent microorganism, whose port of entry is the gastrointestinal tract. The clinical picture of listeriosis often includes gastroenteritis with diarrhea. A similar mechanism may be operative in utero. In our series the two cases with *Listeria monocytogenes* intraamniotic infection had meconium-stained fluid. Other types of organisms using the gastrointestinal tract as the port of entry (i.e., enteroviruses) also may cause in utero meconium passage (Romero R. Unpublished observations).

In this series, it is noteworthy that, except for *Listeria*, the microbial isolates of women with meconium-stained amniotic fluid were not different from those recovered from women with intraamniotic infection and clear am-

Table II. Gestational age on admission, interval to delivery, and birth weight in women with meconium-stained amniotic fluid in association with culture results

	Meconium and positive amniotic fluid culture (n = 10)	*Meconium and negative amniotic fluid culture (n = 20)*	*p Value*
Gestational age (wk)	28.1 ± 4.7	30.4 ± 4.2	0.088
Birth weight (gm)	1400.5 ± 895.3 (370-3190)	2025.0 ± 875.4 (850-4220)	0.075
Interval to delivery (hr)	100.2 ± 222.4 (1-720)	208.5 ± 390.5 (4-1272)	0.434

niotic fluid. We were unable to document a relationship between inoculum size or gestational age and the presence of meconium-stained amniotic fluid. It is not possible to determine whether virulence of a particular strain may play a role.

The increased prevalence of microbial invasion of the amniotic cavity in women with meconium-stained amniotic fluid may be related to alteration of the host-defense mechanism due to the presence of meconium. Amniotic fluid has physiologic antimicrobial properties which are impaired by the addition of meconium.[8, 9] Recently, it has been proposed that the mechanism by which meconium enhances bacterial growth is the alteration of the zinc-to-phosphorus ratio.[10]

Up to this point, our discussion has assumed that the identification of discolored amniotic fluid as meconium stained is accurate. However, the presence of green-stained amniotic fluid may be the result of contamination of amniotic fluid with hemoglobin and its catabolic products. This observation has been made in mid-trimester amniotic fluid and with in vitro experiments.[11] We propose that an ascending intrauterine infection may cause deciduitis and hemorrhage. Passage of hemoglobin and its metabolic by-products into the amniotic fluid may then lead to brown- or green-colored fluid. This postulate is consistent with the observations of Cassell et al.[12] who reported the recovery of *Mycoplasma hominis* and *Ureaplasma urealyticum* in 12% of samples with discolored, second-trimester amniotic fluid.

The prevalence of meconium-stained/discolored amniotic fluid in this study was 4.2%, which is similar to the 5% reported by Ostrea and Naqvi[13] in patients with a gestational age of <38 weeks. Although the frequency of meconium-stained amniotic fluid varies among different reports, there is general agreement that the prevalence is higher in term and postterm pregnancies than in preterm gestations. This finding has been attributed to maturation of the mechanisms responsible for meconium passage.[14]

Another finding of our study is that patients with meconium-stained/discolored amniotic fluid were more likely to fail tocolysis and to deliver prematurely than women with clear fluid. This association remained highly significant even after women with intraamniotic infections were removed from the data set (75% [15/20] vs 30.2% [183/602]; $p = 0.00007$; odds ratio = 6.8; 95% confidence interval = 2.3 to 21.9). In this setting the presence of meconium-stained/discolored amniotic fluid may be a clinical marker for pathologic processes associated with preterm labor (i.e., fetal distress, abruptio placentae, etc.).

The clinical significance of this report is the identification of meconium-stained/discolored amniotic fluid as a risk factor for microbial invasion of the amniotic cavity and preterm delivery. This study has focused on the preterm gestation. Given the high prevalence of unexplained meconium passage in term and postterm gestations and the seriousness of its sequelae, the possible association between intraamniotic infection and meconium-stained fluid at term must be examined.

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Mild fetal lateral cerebral ventriculomegaly: Clinical course and outcome

Bryann Bromley, MD, Fredric D. Frigoletto, Jr., MD, and Beryl R. Benacerraf, MD
Boston, Massachusetts

The neonatal, pathologic outcome and karyotypic abnormalities are reported for 44 fetuses with mild ventriculomegaly diagnosed antenatally. Seventeen of these 44 fetuses (39%) had other ultrasonographic defects, and five (12%) had abnormal karyotypes. Five pregnancies were electively aborted and three other fetuses died in the neonatal period. Twenty-six (72%) of the remaining 36 live-born neonates are developmentally and clinically normal at 3 to 18 months of age. Twenty-one of these 26 had isolated mild ventriculomegaly as the only ultrasonographic finding. The other 10 live-born infants are developmentally impaired, and five of these 10 had mild ventriculomegaly as the only prenatal ultrasonographic abnormality. In conclusion, these data show that fetuses with mild ventriculomegaly have a lower incidence of associated anomalies and a better outcome than fetuses with more severe ventricular dilatation, as reported in the literature. The majority of fetuses with mild ventriculomegaly as an isolated finding and a normal karyotype are developing normally. (Am J Obstet Gynecol 1991;164:863-7.)

Key words: Ventriculomegaly, karotype, anomaly

Congenital ventriculomegaly is a serious malformation of the central nervous system occurring in one per 1000 fetuses. It is often associated with a poor prognosis.[1] Prenatal ultrasonography accurately detects fetal central nervous system anomalies, including ventriculomegaly, permitting counseling of expectant parents concerning predicted outcomes.[2-6] Estimation of ventricular size has become an important part of the prenatal ultrasonogram in the assessment of brain development. The atria of the normal lateral vetricles remain relatively constant in size throughout gestation, with a mean diameter of 7.6 ± 0.6 mm.[7, 8] Ventriculomegaly is suspected when the atrial diameter reaches 10 mm.[7, 3] Although the outcome of severe ventriculomegaly is poor and has been extensively studied, the natural history and significance of mild or borderline ventriculomegaly is unknown. We report the associated congenital and karyotypic abnormalities and clinical outcome in 44 patients with mild ventriculomegaly (atrial measurement 10 to 12 mm) diagnosed antenatally with ultrasonography.

Material and methods

Mild ventriculomegaly was considered present when the axial ultrasonogram of the brain demonstrated an atrial diameter of the lateral ventricle between 10 and 12 mm. Forty-nine consecutive fetuses between 16 and 37 weeks' gestation, scanned between February 1987 and May 1990 and delivered by June 1, 1990, met this criterion (Fig. 1). Clinical follow-up was available in 44 patients by review of the pediatric record, consultation with the pediatrician, and discussion with the parents. Five patients were unavailable for follow-up or had inadequate or incomplete information and were excluded from the study.

Each scan was reviewed retrospectively; an image of the atrium of the lateral ventricle was available in each case. The atrium of the lateral ventricle had been photographed prospectively as part of the scan and was retrospectively measured and analyzed in each case. If the lateral ventricular atrium measured between 10 and 12 mm, the patient was included in the study regardless of indication for the scan. Other findings tabulated included the assessment of anmiotic fluid volume and evidence of other congenital malformations at the time of the original ultrasonogram. Each scan was done with an Acuson 128 variable-focus 3.5 MHz sector transducer; each patient was scanned by a single sonologist. Clinical circumstance dictated interval of ultrasonographic follow-up examinations.

Results

The ultrasonographic diagnosis of mild ventriculomegaly was made in 49 consecutive fetuses between the gestational ages of 16 and 37 weeks (Fig. 2). Forty-four patients had adequate follow-up of their neonates between 3 and 18 months of age. All patients consecutively noted to have mild ventriculomegaly (atrium of the lateral ventricle between 10 and 12 mm, Fig. 1)

From the Departments of Obstetrics and Gynecology and Radiology, Brigham and Women's Hospital, Harvard Medical School.
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Reprint requests: Bryann Bromley MD, Diagnostic Ultrasound Associates, 333 Longwood Ave., Boston, MA 02115.
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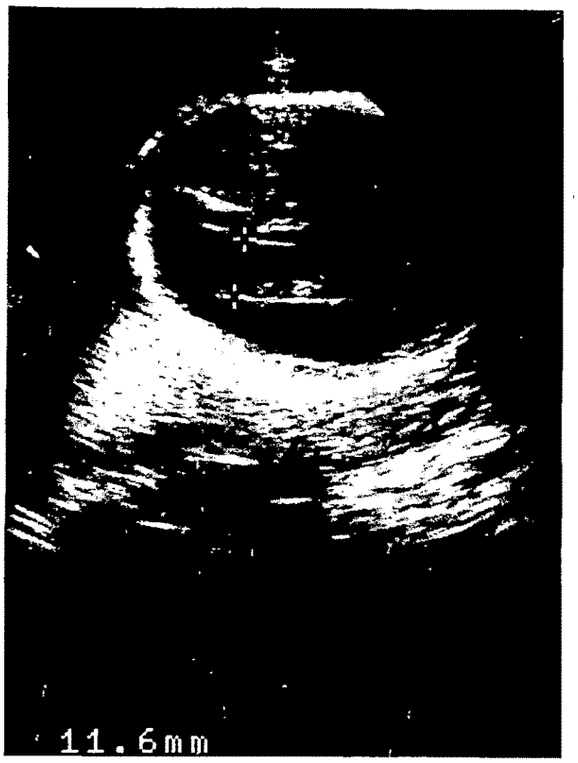


Fig. 1. Transverse view of the fetal head showing mild ventriculomegaly. Width of atrium of lateral ventricle is 11.6 mm.

Table I. Indication for scans

Indication for scan	*No. of patients*
Suspected central nervous system defect	13
Suspected non–central nervous system defect or polyhydramnios	8
Abnormal α-fetoprotein	2
Low risk for ventriculomegaly	21
TOTAL	44

were included in the study, regardless of indication for the ultrasonogram. Mild ventriculomegaly was identified ultrasonographically in 21 patients at low risk for having central nervous system abnormalities. There were 13 patients who were referred to our facility because of suspected ventriculomegaly on a level I ultrasonogram, two others because of an abnormal α-fetoprotein value, and eight more who were believed to have an abnormality not related to the central nervous system (Table I).

Seventeen of the 44 fetuses (39%) with mild ventriculomegaly had other abnormalities detected ultrasonographically. Six of these had other central nervous system anomalies and 11 had non–central nervous system defects (Table II).

Table II. Other malformations (total 17 patients*)

	No. of patients
Central nervous system defects	
Neural tube defect	3
Agenesis of the corpus callosum	1
Subarachnoid cyst	1
Hypoplastic cerebellum (trisomy 18)	1
Anomalies outside central nervous system	
Omphalocele	1
Abnormal extremities	4
Heart defects	4
Transient ascites	2
Facial cleft	2
Hydrops	1
Duodenal atresia	1

*Several fetuses appear in more than one category.

Twelve patients underwent cytogenetic studies at the discretion of the referring obstetrician because of advanced maternal age, abnormal α-fetoprotein level, or multiple fetal anomalies. Five of these amniocenteses revealed an abnormal karyotype: Three fetuses had trisomy 21, one had trisomy 18, and one had a 7p- karyotype. All five fetuses with karyotypic abnormalities had other abnormal ultrasonographic findings, although the patient with the 7p- karyotype had coarctation of the aorta, which was difficult to detect ultrasonographically. The fetuses with trisomy 21 had hydrops, duodenal atresia, cardiac defects, and intrauterine growth retardation. Although not all fetuses actually had karyotyping done, those who did not undergo amniocentesis did not show any clinical suggestion of aneuploidy after birth. Only one patient who underwent pregnancy termination for neural tube defect did not have karyotypic information either in utero or at termination (Table III). The overall incidence of abnormal karyotype in our study therefore was five of 43 (12%).

Of our 44 patients, 32 had follow-up scans in utero, with normalization of the lateral ventricular measurement in five. Four of these five neonates exhibited normal development at 6 to 15 months of life. The other infant had congenital glaucoma. These five fetuses were initially diagnosed as having mild ventriculomegaly (atrium, 10 to 12 mm) between 27 to 32 weeks' gestation, and had normalized (atrium, ≤9 mm) by 33 to 37 weeks.

Twenty-three patients had postnatal radiologic or pathologic follow-up revealing abnormal intracranial findings in 11 patients. These follow-up scans were done at the discretion of individual pediatricians. None of the neonates required shunting.

Five pregnancies ended in elective abortion and three

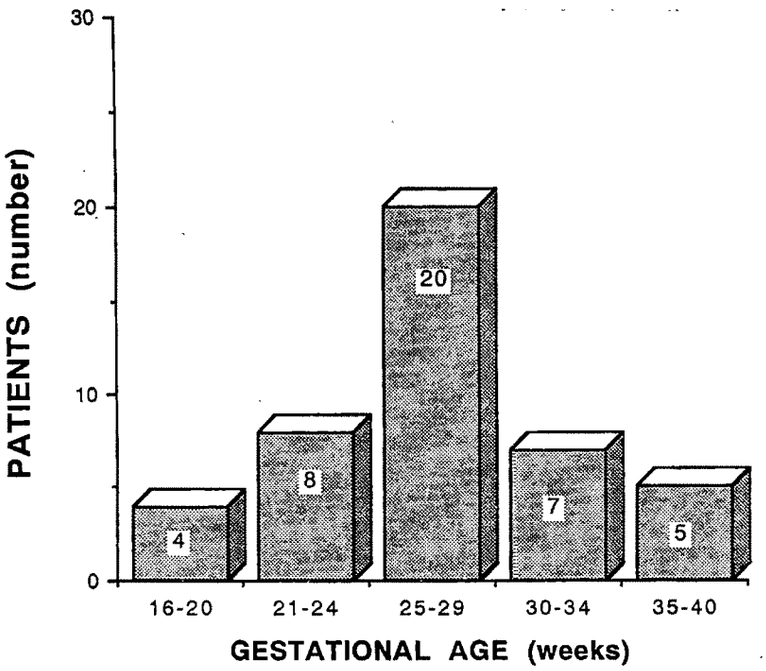


Fig. 2. Graph showing gestational age of fetuses with mild ventriculomegaly.

Table III. Fetal deaths (total 8 patients)

	Karyotype
Elective abortion (5 patients)	
Isolated ventriculomegaly	Normal amniocentesis
Neural tube defect (2 patients)	One with normal amniocentesis
Abnormal distal limbs	Normal amniocentesis
Trisomy 21	Trisomy 21
Fetal or neonatal death (3 patients)	
Heterotaxy syndrome with heart defect	Normal karyotype
Trisomy 21 with hydrops	Trisomy 21
Trisomy 18 with multiple anomalies	Trisomy 18

had intrauterine fetal death or neonatal death. The associated pathologic conditions of these eight losses are shown in Table III.

Developmental information was available in all 36 pregnancies with living children by reports from pediatricians or pediatric neurologists in the majority of cases, as well as by interviews with parents (Fig. 3). Twenty-six neonates were reported to be clinically and developmentally normal at 3 to 18 months of age. Ten patients (28%) were clinically or developmentally impaired, as shown in Table IV. These 10 infants were all considered impaired by their pediatricians or neurologists using standard pediatric assessment.

Isolated ventriculomegaly with no other congenital defect was noted in 27 of the total 44 patients, and 21 of these are developing normally. Five of the 27 with isolated ventriculomegaly are handicapped (hearing impaired, congenital glaucoma, congenital seizures, and two with mild delay in motor development). One underwent pregnancy termination. From a different viewpoint, 26 of the total 44 neonates are developing normally; of these, 21 had isolated ventriculomegaly. The five neonates developing normally with other ultrasonographic findings included one with agenesis of the corpus callosum, one with an omphalocele, one with a cleft lip and palate, and two with transient ascites. From yet another perspective, five of the 10 fetuses with abnormal developmental outcomes had no other ultrasonographic defects and five had other congenital abnormalities, including one each with a neural tube defect, subarachnoid cysts, and clubbed feet and two with congenital heart defects (both of whom had abnormal karyotypes).

There were 13 of 44 pregnancies (30%) that were complicated by polyhydramnios, seven of which had other ultrasonographically identified defects. Three of

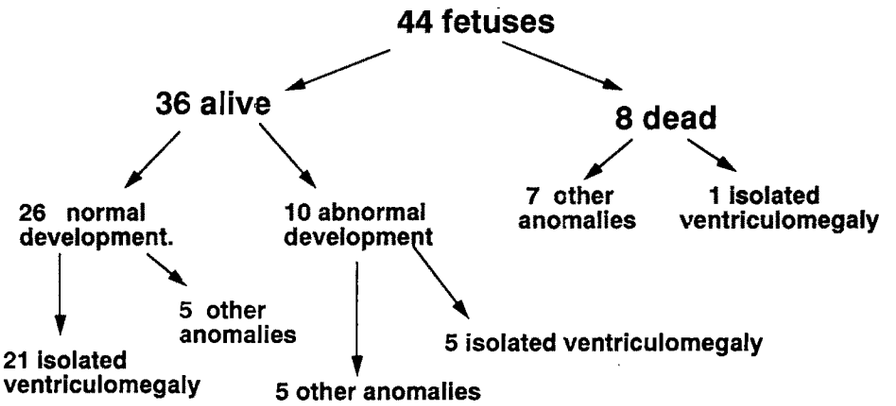


Fig. 3. Flow chart of developmental outcome of 44 fetuses with mild ventriculomegaly.

Table IV. Developmental impairment (10 patients)

Abnormality	*No. of patients*
Hearing loss	1
Neonatal glaucoma	1
Infantile seizures	1
Delayed motor development (normal karyotype)	2
Neural tube defect	1
Myotonic dystrophy	1
Aicardi syndrome	1
Down syndrome	1
Karyotype 7p-	1

these 7 fetuses died and one other is developing abnormally. The other nine neonates are developing normally.

In conclusion, of the total 44 patients with clinical follow-up, 26 of 44 (59%) of the infants were developing normally, 12% had abnormal karyotypes, and 18% were either electively aborted or stillborn. Of the 36 live births, 26 of 36, or 72%, resulted in developmentally normal neonates.

Comment

Fetal intracranial ventricular enlargement has been associated with a poor prognosis, regardless of cause.[2-6] Previous series have shown that the majority of fetuses with significant ventriculomegaly have associated major abnormalities and an unfavorable outcome. Vintzileos et al.[2] reported associated abnormalities in 70% of fetuses with ventriculomegaly. Only 45% of the neonates in his study were alive, but more than half of these were developmentally impaired. Chervenak et al.[3] described 53 fetuses with enlarged ventricles, 83% of whom had other developmental abnormalities, with only 28% survival. Nyberg et al.[4] also reported that a majority of fetuses with ventriculomegaly had other congenital abnormalities; this was associated with a universally fatal outcome in their series. Pretorius et al.[5] found the prognosis of fetuses with enlarged intracranial ventricles to be poor, with only 15% survival at 13 months of age and half of those developing abnormally. This is in agreement with the work of Chervenak et al.,[3] which showed that in a series of fetuses with ventriculomegaly there were associated abnormalities in 85% of cases.

Mahony et al.[9] reported on 20 fetuses with mild dilatation of the lateral ventricles. In their study 40% of fetuses with ventriculomegaly had a normal outcome, 40% of fetuses died, and 20% had an uncertain prognosis. Their criteria for ventriculomegaly was based on the distance between the medial aspect of the lateral ventricular atrium and medial surface of the choroid plexus, which is not a well-established landmark for describing hydrocephalus. In their patients the atrial diameter was between 9 and 15 mm, which is a wider span than reported here. We considered an atrial measurement of >12 mm not to represent mild ventriculomegaly.

A recent study by Goldstein et al.[10] describing 55 cases of mild ventriculomegaly showed that isolated mild ventricular dilatation had a better prognosis than nonisolated ventriculomegaly. However, they included atrial measurements up to 15 mm, which we believe is more than mild ventricular dilatation. Also, their mortality figures (40 of 55 fetuses, or 73%) were much higher than ours, presumably because many patients underwent termination of pregnancy (30 of 55 patients).

A dilemma exists when ventriculomegaly is only mild or when the ventricles are borderline in size. Although it is not a challenge to diagnose severe ventriculomegaly in utero, it may be difficult for the ultrasonologist to decide whether minimal ventriculomegaly or borderline atrial enlargement represents a true abnormality. The prenatal diagnosis of ventriculomegaly has devastating implications in terms of fetal survival and infant development, which may alter the management of the pregnancy, thereby influencing outcome. Cardoza

et al.[8] reported that the mean diameter of the normal atrium of the lateral ventricles is constant throughout gestation and measures 7.6 ± 0.6 mm. These authors considered an atrial width >10 mm to be abnormal. Although it is clear that gross ventriculomegaly indicates a true abnormality, the atrial ventricular size between 10 and 12 mm is a gray area and the outcome of affected fetuses has not previously been investigated systematically. It is difficult to know how to counsel a patient whose fetus has ventricular measurements in this borderline area; for example, there have been two case reports of resolution of mild ventriculomegaly, which may have represented fetuses in this intermediate zone.[11, 12]

Our data show that fetuses who have lateral ventricular measurements betweeen 10 and 12 mm have a better outcome than those reported in the literature who have obvious ventricular enlargement. In our study 26 of 44 (59%) of all the neonates are developing normally, and of those with isolated ventriculomegaly whose pregnancy was not electively terminated, 21 of 26 (80%) resulted in normal neonates, a much better outcome than in previously reported studies of ventriculomegaly. Only 17 (39%) of the total of 44 fetuses had other anomalies identified, which is a lower rate of associated anomalies than reported with more severe ventricular enlargement.[3-6]

However, not all fetuses with isolated ventriculomegaly had good outcomes. Of the 10 fetuses with abnormal development, half had isolated ventriculomegaly and the other half had other congenital defects. Conversely, although the majority of the 26 fetuses developing normally had isolated ventriculomegaly, five of these 26 clinically normal fetuses had other congenital defects demonstrated ultrasonographically (one omphalocele, one agenesis of the corpus callosum, one facial cleft, and two transient ascites).

A limitation of this study was that the neurologic examination was done by individual pediatricians or pediatric neurologists and was not standardized. However, the clinical data from each of the 10 children considered impaired were obtained directly from the pediatrician or pediatric neurologist. All other neonates (considered normal) had normal results of pediatric examination and frequently normal head ultrasonograms.

In summary, although 80% of fetuses with isolated ventriculomegaly are developing normally, 50% of those with other congenital defects showed developmental impairment, suggesting that the detection of additional structural defects worsens the prognosis.

Five (12%) of 43 had abnormal karyotypes, indicating that cytogenetic studies should be considered when mild ventriculomegaly is detected ultrasonographically, particularly if other abnormalities are visualized. Although actual karyotypes were available on 12 patients, all those with abnormal karyotypes had other ultrasonographic findings besides ventriculomegaly, putting them at higher risk of aneuploidy than those with isolated ventricular dilatation. Furthermore, there was no clinical evidence of aneuploidy among the remaining 31 infants (one additional patient with a fetus having a neural tube defect aborted with inadequate information regarding chromosomes). It is of note that two of the fetuses with abnormal chromosome complements in our study had subtle cardiac anomalies that might not always be recognized ultrasonographically unless formal echocardiography is done.

In conclusion, there appears to be a subset of fetuses with mild ventriculomegaly who may have fewer associated anomalies and a better prognosis than those more severely affected. Although the lateral ventricular atrial width of ≥10 mm is definitely abnormal, the majority of fetuses with this measurement as an isolated finding in the setting of a normal karyotype are developing normally. When mild ventriculomegaly is detected ultrasonographically, karyotypic studies and follow-up scans are recommended. It is clear that further studies will be necessary to study long-term development of these children. However, the presence of mild ventriculomegaly seems to be associated with a more optimistic outcome than moderate or severe ventricular enlargement.

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Hospital setting and fetal death during labor among women at low risk

Leah L. Albers, DrPH,[a] and David A. Savitz, PhD[b]
Chapel Hill, North Carolina

Intrapartum fetal death in low-risk women at term is a rare obstetric outcome. This is difficult to study because few data sets contain an adequate number of cases for meaningful analysis. This study used data from the 1980 National Natality Survey and National Fetal Mortality Survey, merged with an American Hospital Association annual survey for the same year, to determine whether the frequency of intrapartum fetal death in low-risk women varied by the hospital setting for birth. Stratified analysis was used to assess the relation of level of hospital for delivery with intrapartum fetal death, with control for potential confounding factors. As the level of available perinatal technology decreased, the frequency of intrapartum fetal death increased (odds ratio, 2.0 for Level II and 3.3 for Level I, as compared with Level III hospitals). Even when early neonatal deaths were considered, perinatal mortality remained lowest at Level III facilities (odds ratio, 1.6 for Level II and 2.7 for Level I, as compared with Level III hospitals). The components of intrapartum surveillance that are most effective in the reduction of perinatal mortality have not been identified. (AM J OBSTET GYNECOL 1991;164:868-73.)

Key words: Obstetrics, hospital level, intrapartum fetal death

Avoidance of fetal death remains an important concern of perinatal health professionals. Since the late 1970s, perinatal mortality has been made up of proportionately more fetal than neonatal deaths.[1] The risk of fetal death is known to decline as gestation advances,[2] and fetal death occurs with increased frequency at the extremes of reproductive age,[1,2] in women of high parity,[3] those with medical problems,[2,4] smokers,[5] and the socially disadvantaged.[6]

Most fetal deaths occur during the antepartum period, before the onset of labor. Large vital records series have shown that in pregnancies beyond 24 weeks' gestation, 12.8% of fetal deaths occur during labor.[3] Intrapartum fetal death is thought to be sensitive to the level of obstetric care,[7] with the frequency declining as the technical sophistication of the hospital for delivery is increased. If lethal anomalies are excluded, intrapartum fetal death is presumed to be largely caused by maternal or placental factors that may be amenable to medical intervention.[4] This opportunity for successful intervention is less apparent for fetal deaths that occur before labor begins.

Women at low risk constitute a large group that uses considerable health resources, particularly given essentially universal hospitalization for birth in the United States. Low-risk women are delivered in all hospital settings, because distance and travel time strongly influence the choice of hospital for this group.[8] For low-risk pregnancies at term, newborn survival is not improved by delivery in a Level II or III hospital, as compared with a Level I setting.[9,10] The relation of the birth setting to the other component of perinatal mortality in low-risk women, intrapartum fetal death, has not to our knowledge been explicitly addressed previously.

Like other infrequent pregnancy outcomes, intrapartum fetal death in low-risk women is difficult to study because few data sets contain an adequate number of cases for meaningful analysis. Although rare, intrapartum fetal death in low-risk women is a very important outcome because of the powerful emotional and psychological sequelae it generates for affected families, as well as the medical liability concerns for care providers and institutions.

The purpose of this study was to measure the frequency with which intrapartum fetal death occurs in low-risk pregnancies in the United States, and to determine whether this outcome varies according to the hospital setting for delivery.

Methods

Data source. A tape in which the 1980 National Natality Survey and National Fetal Mortality Survey are linked with an American Hospital Association annual survey of U.S. hospitals for the same year was provided as an in-house data tape, which is the property of the National Center for Health Statistics. The National Natality Survey and the National Fetal Mortality Survey sampled live births and fetal deaths from all 52 regis-

From the Departments of Maternal and Child Health[a] and Epidemiology,[b] University of North Carolina School of Public Health.
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Reprint requests: Leah L. Albers, DrPH, Rt. 9, 2 Jones Branch, Chapel Hill, NC 27514.
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tration areas in the United States during 1980. The sampling methods and rationale are explained in detail elsewhere.[11] The sample contains 9941 live births, which represent 3,612,258 for 1980 for a sampling fraction of 1 in 363, and 6386 fetal deaths, which represent 19,202 during that year for a sampling fraction of 1 in 3. Fetal deaths were defined as those with a gestation of ≥28 weeks, or a delivery weight of at least 1000 gm. In the National Natality Survey, low-birth-weight babies were oversampled so that roughly four times the number that would have been chosen by simple random sampling were included.

Information was collected from the live birth-fetal death certificates, and then from questionnaires sent to married mothers, the hospitals of delivery, and health care providers. Data from the vital records were very nearly complete, but the mothers (20.5% in the National Natality Survey, and 25.5% in the National Fetal Mortality Survey) and the hospitals (23.9% in the National Natality Survey and 26% in the National Fetal Mortality Survey) had larger proportions of missing data. Missing entries were completed from another source when possible, and otherwise imputed by the use of specific combinations of selected predictor variables.[11] For this study, imputed data for the outcome of interest (fetal death in labor) are regarded as missing. Imputed data for the control variables were included in the study. Data in the American Hospital Association annual survey[12] allowed the examination of the outcome of labor (fetal death vs. live birth) according to certain hospital characteristics.

Study sample. The study sample was restricted to low-risk women at term because medical or obstetric conditions, as well as preterm or postterm status, may increase the likelihood of intrapartum fetal death and may also influence the choice (by the woman or her health care provider) of the hospital setting for delivery. Low-risk women are presumed to choose hospitals for reasons other than known indications for particular types of care.

Women in the National Natality Survey and the National Fetal Mortality Survey who met all the following restriction criteria constituted the low-risk study sample: gestational age, 37 to 42 weeks; maternal age, 18 to 35 years; first to fourth baby; 8 years or more of education; prenatal care beginning the first to fifth month and including at least five visits; absence of obstetric problems (breech or transverse lie, multiple gestation, hypertensive disorders); and absence of preexisting medical problems (cardiovascular, pulmonary, or renal disease; diabetes; obesity; Rh disease; sickle cell anemia; drug abuse; or alcoholism). We also excluded births that occurred out of hospital, in a hospital with no birth service, or in a hospital with no data in the American Hospital Association survey. The final sample contained 4477 live births, representing 1,821,127 in the United States for 1980, and 183 intrapartum fetal deaths, representing 556 nationally among low-risk women that year.

Hospital level. Hospital level was assigned by applying criteria from the Regionalized Perinatal Care program[13, 14] to variables in the American Hospital Association portion of the merged tape. The following definitions were used:

Level III: a teaching hospital (medical school affiliated, with American Medical Association–approved residency training programs) with a neonatal intensive care unit on-site, or a teaching hospital that performs at least 2000 deliveries annually with no on-site neonatal intensive care unit but with a formal contractual arrangement for neonatal intensive care services at a nearby facility

Level II: other urban hospitals

Level I: other rural hospitals (located in nonmetropolitan areas).

Choice of hospital for delivery is acknowledged to be nonrandom, but is primarily a function of geographic proximity if no extenuating medical or financial circumstances apply.[8]

A clear gradient of the available perinatal technology across hospital level is shown in Table I. As expected, the Level III facilities were larger, had a higher volume of deliveries, and had greater nursery capabilities. The mother's place of residence (rural-urban) and the location of the hospital for delivery were strongly associated.

Outcome. Intrapartum fetal death for this restricted group of low-risk women at term was the outcome of interest. Data were excluded for fetal deaths if death was not known with certainty to have occurred during labor.

Control variables. Ten extraneous variables that are related to intrapartum fetal death and that could vary by hospital setting and thus obscure the hospital setting–labor outcome relationship for the low-risk sample, were considered. No significant differences in number of prenatal care visits, duration of labor, labor complications, birth defects, and birth weight were found across hospital level. For maternal age, parity, maternal education, race, and marital status, small differences were found, but these were not of sufficient magnitude to confound the relationship of hospital setting to the outcome of labor to any important extent.

Analysis. For the analyses, deliveries in Level III settings were considered the reference group, the risk of fetal death in labor being lowest here. Mantel-Haenszel odds ratios and test-based 95% confidence intervals were calculated through stratified analysis for

Table I. Description of hospitals by level

	Hospital level		
	I	*II*	*III*
Newborn intensive care unit (%)	19.9	23.4	93.5
Intermediate nursery (%)	54.4	69.0	94.2
Medical school affiliation (%)	2.0	16.3	100.0
AMA residencies (%)	3.9	26.4	100.0
No. of beds (%)			
<100	38.6	6.0	0.0
100-299	53.5	46.4	7.6
300+	7.9	46.7	92.4
No. of births per year (%)			
500 or less	44.2	8.7	0.0
501-2,000	53.3	64.0	22.9
>2,000	2.5	27.3	77.1
Mean No. of newborn intensive care unit			
In-patient days	55.2	155.1	3062.7
Discharges	3.2	14.2	226.5
Location of mother's residence			
Urban (%)	3.2	85.1	88.8
Rural (%)	96.8	14.9	11.2

the association of hospital level with intrapartum fetal death, comparing Level II with III and Level I with III. Crude odds ratios were compared with those adjusted individually for dichotomized control variables, to assess whether any were capable of distorting the hospital setting-labor outcome relationship.

To examine total perinatal mortality in low-risk women (including neonatal deaths), data from the 1980 National Natality Survey and the National Death Index Match Project[15] were used. This is National Center for Health Statistics data in which sampled births from the 1980 National Natality Survey were followed up in 1980 and 1981 to ascertain whether any infant deaths were reported. Sample weights were also applied to live births and intrapartum fetal deaths in the 1980 National Natality Survey and the National Fetal Mortality Survey to derive intrapartum mortality ratios (the number of fetal deaths in labor per 1000 live births) for low-risk women in the United States.

Statistical analysis was carried out with an IBM mainframe computer and software from the SAS Institute[16] at the Research Triangle Park Branch of the National Center for Health Statistics.

Results

Compared with low-risk deliveries in the Level III settings, the risk of intrapartum fetal death at Level II hospitals was twofold higher, and at Level I hospitals was 3.3-fold higher (Table II). Adjustment for the 10 dichotomized extraneous variables indicated an absence of confounding, with odds ratios after adjustment remaining consistent with the crude odds ratios. For both comparisons, slightly stronger associations were seen for primigravidas. In the Level I versus III comparison, stronger associations were seen for unmarried women and for normal birth weight babies.

To examine whether the lowered intrapartum fetal deaths in Level III settings might actually represent a postponement of death to the early newborn period (first week of life), data from the 1980 National Natality Survey and the National Death Index Match Project were examined. Of the 9941 sample births in the 1980 National Natality Survey, 295 infants died in the first year of life. Of the 4477 low-risk births in the National Natality Survey, 24 infants died during the first year, of which 16 deaths occurred in the first week of life. These were distributed as four each for Level I and II hospitals, and eight for Level III hospitals. Odds ratios and 95% confidence intervals that compare intrapartum fetal deaths and perinatal deaths (deaths occurring from labor through the first week of life) are shown in Table III. Even after consideration of early neonatal deaths, perinatal mortality for low-risk women remained lowest at Level III hospitals.

Finally, sampling weights were applied to live births and intrapartum fetal deaths in the 1980 National Natality Survey and the National Fetal Mortality Survey to derive intrapartum mortality ratios (the number of fetal deaths in labor per 1000 live births) for low-risk women in the United States (Table IV). For low-risk women in 1980, intrapartum fetal death occurred roughly once in 3300 deliveries. If these events for Level I and II are combined (474 of 1,288,820) and then compared with the ratio for Level III, the differ-

Table II. Summary of stratified analysis

Stratification variables	Level II vs III*				Level I vs III†			
	Stratum-specific odds ratio	*95% CI*	*Adjusted odds ratio*	*95% CI*	*Stratum-specific odds ratio*	*95% CI*	*Adjusted odds ratio*	*95% CI*
No. of prenatal care visits								
≥15	1.9	1.2-3.0	2.0	1.3-3.0	3.8	1.0-14.9	3.4	2.2-5.1
<15	2.3	0.7-8.4			3.3	2.0-5.3		
Duration of labor (for parity)								
>Median	1.7	0.9-3.3	2.0	1.3-3.0	3.6	1.8-7.0	3.3	2.2-5.1
≤Median	2.2	1.2-3.9			3.2	1.7-5.8		
Labor complications								
Yes	2.3	1.1-5.0	2.0	1.3-3.0	3.9	1.8-8.5	3.4	2.2-5.2
No	1.8	1.0-3.0			3.2	1.8-5.5		
Birth defects								
Yes	1.9	0.8-4.6	2.0	1.3-3.1	1.9	0.7-5.1	3.3	2.2-5.1
No	2.0	1.2-3.4			3.8	2.3-6.4		
Birth weight								
≤2500 gm	1.3	0.6-3.1	2.0	1.3-3.1	1.5	0.6-3.9	3.5	2.3-5.3
>2500 gm	2.3	1.4-4.0			4.3	2.5-7.4		
Maternal age								
30-35 (yr)	2.2	0.9-5.6	2.0	1.3-3.0	3.5	1.3-9.9	3.4	2.2-5.2
18-29 (yr)	1.9	1.2-3.1			3.4	2.0-5.6		
Parity								
Primigravid	2.7	1.4-5.5	2.0	1.3-3.0	4.4	2.1-9.2	3.3	2.2-5.1
Multigravid	1.5	0.9-2.7			2.8	1.6-4.9		
Education								
>12 yr	1.7	0.9-3.4	2.0	1.3-3.0	3.1	1.5-6.3	3.4	2.2-5.2
≤12 yr	2.1	1.2-3.8			3.5	2.0-6.4		
Race								
White	2.2	1.3-3.7	2.0	1.3-3.1	3.8	2.2-6.3	3.4	2.2-5.3
Nonwhite	1.2	0.5-3.3			2.1	0.7-6.1		
Married								
Yes	2.1	1.3-3.5	2.0	1.3-3.0	3.3	2.0-5.4	3.5	2.3-5.3
No	1.2	0.4-3.7			4.6	1.7-12.5		

CI, Confidence interval.
*Crude odds ratio, 2.0; 95% confidence interval, 1.3=3.0.
†Crude odds ratio, 3.3; 95% confidence interval, 2.2=5.1.

ence implies that approximately 5000 low-risk women would have to be delivered outside a Level III facility before one excess intrapartum fetal death would occur.

Comment

Intrapartum fetal death is a rare obstetric outcome, particularly for low-risk women at term. Nonetheless, if lethal anomalies are excluded, it may be at least partially preventable. This analysis showed that as the level of available perinatal technology decreased, the frequency of intrapartum fetal death in women at low risk increased. Characteristics of the childbirth setting (hospital size, volume of deliveries, teaching status, increased nursery capabilities) influenced the risk of intrapartum fetal death, even among low-risk women.

The lowered incidence of intrapartum fetal death in settings with a higher obstetric volume may indicate that medical care providers and nursing staff are able to maintain skill levels. In teaching centers, oversight or supervision mechanisms (case reviews, second opinions) may be more institutionalized. Urban hospitals may be in a more favorable position to recruit staff nurses and may have higher nurse-patient ratios for obstetrics, given that a substantial proportion of the deliveries are high risk. In small hospitals with fewer deliveries, the obstetric volume may not justify continuous staffing for a labor unit, and nurses may be moved from other clinical areas when a labor patient is admitted. In these situations, knowledge of and experience with labor surveillance may be more uneven.

This study has several limitations that require caution in interpreting the observed associations as causal. First, if unmeasured risks for intrapartum fetal death influenced hospital choice, the results would be biased. Second, our study may be in part addressing rural-urban differences. Women who reside in rural areas are known to be poorer, have reduced access to health care providers and institutions, and have higher fetal death rates.[17] Data on physician specialty (obstetrics and

Table III. Mortality odds ratios by hospital level

	Level II vs III		*Level I vs III*	
	Odds ratio	*95% CI*	*Odds ratio*	*95% CI*
Intrapartum fetal death	2.0	1.3-3.0	3.3	2.2-5.1
Perinatal death	1.6	1.1-2.3	2.7	1.8-4.0

CI, Confidence interval.

Table IV. Intrapartum mortality ratios for low-risk women

Hospital level	*Live births*	*Intrapartum fetal death*	*Ratio*
III	532,307	82	0.15/1,000
II	863,120	259	0.30/1,000
I	425,700	215	0.51/1,000
TOTAL	1,821,127	556	0.31/1,000

gynecology or family medicine) are not available in the 1980 National Natality Survey and the National Fetal Mortality Survey. However, the relative predominance of family physicians as providers of obstetric care in rural areas is well known.[18] Third, lack of other information also limited the analysis. The data on birth defects did not permit lethal anomalies to be dropped from the analysis, and deletion of such could alter our odds ratios, although not in a predictable direction. The role of electronic fetal monitoring could not be elucidated, although two large randomized trials[19, 20] previously showed no reduction in the incidence of intrapartum fetal death by routine use of electronic monitoring. Electronic fetal monitoring was more common in cases of intrapartum fetal death than in live births (52.5% compared with 46.5%), but these data did not report the point in labor when electronic fetal monitoring was applied, or the reason for its use. Electronic fetal monitoring could have been used after the fact, to confirm fetal death. Data were also lacking on nursing care patterns. Staffing practices of obstetric nurses, nurse-patient ratios, and protocols for intermittent auscultation of the fetal heart rate would have enriched the analysis.

A final limitation is the age of the data. Changes in obstetric practice during the 1980s because of factors such as the availability and use of technology, referral patterns, the medical liability insurance crisis, and the resultant decrease in obstetric providers, particularly in rural areas, or even altered characteristics of low-risk women, might yield different results for intrapartum fetal death now.

It is of interest that the primary cesarean delivery rate for cases of intrapartum fetal death was threefold higher than for live births (28.9% compared with 10.4%) and did not vary significantly by hospital level. This decreases the likelihood that these fetal deaths occurred in the antepartum period but were incorrectly coded as intrapartum deaths. This distinction is important because reporting accuracy can strongly influence correct measurement of this rare obstetric outcome.

The proper conclusion to be drawn from our results is not that all low-risk women should be delivered in Level III hospitals, a suggestion that is neither practical nor affordable for the nation, but rather that the components of appropriate and effective intrapartum surveillance for low-risk women, characteristic of Level III hospitals, need to be delineated. Specific clinical practices, the way care is rendered, and institutional assurance mechanisms are all areas that deserve more scrutiny.

In 1988, the National Center for Health Statistics again sampled vital records for the entire U.S. The National Maternal and Infant Health Survey is scheduled to become available to researchers late in 1991. An assessment of the hospital setting–labor outcome relationship for low-risk women and an examination of time trends will then be possible with more current national data.

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Fetal ovarian cysts: Prenatal ultrasonographic detection and postnatal evaluation and treatment

Israel Meizner, MD,[a] Arie Levy, MD,[a] Miriam Katz, MD,[a] Abraham Jacob Maresh, MD,[b] and Marek Glezerman, MD[a]

Beer-Sheva, Israel

Ovarian cysts were diagnosed by antenatal ultrasonographic examination in 15 fetuses between 19 and 37 weeks' gestation. In six cases there was ultrasonographic evidence of torsion. Intracystic flocculation, which typically was deposited on the sloping part of the cyst, gave a characteristic liquid interface that was regarded as ultrasonographic evidence of torsion. All cases with evidence of torsion were managed surgically post partum, and in all patients this complication was confirmed. The remaining nine cases were followed up by repeated ultrasonograms, and in all patients disappearance of the cyst was documented within the first 6 months of life. The mean size of cysts with evidence of torsion was 5.41 ± 0.25 cm, and the mean size of those without torsion was 4.33 ± 0.3 cm ($p < 0.01$). Histologic examination of the surgical specimen in the cases with evidence of torsion revealed follicular cysts in three cases and necrotic ovarian cysts with no specific epithelial findings in the remaining three. We recommend continuous ultrasonographic assessment of antenatally diagnosed cysts and believe that the choice of treatment depends on the appearance of the cyst and its evolution throughout pregnancy. (Am J Obstet Gynecol 1991;164:874-8.)

Key words: Ovarian cyst, torsion, ultrasonography

Small ovarian cysts in the newborn are not uncommon. DeSa[1] found small follicular cysts in 113 (34%) of 332 stillborns or infants who died within 28 days of birth. Doshi et al.[2] reported similar findings in 32% of 40 neonates at autopsy in their series. Most of these small cysts involute within the first few months of life and have no clinical significance. Occasionally, however, a large ovarian cyst is seen as an abdominal mass in the newborn. Because of progress in ultrasonography, it has become possible to assess the antenatal evolution of these cysts. Timely alertness of pediatric surgeons will then permit early neonatal intervention. Only a few cases of antenatal ultrasonographic diagnosis of fetal ovarian cysts have been reported,[3-6] but the therapeutic aspects in these cases have been rarely documented. We present our experience with the diagnostic and therapeutic aspects of antenatally diagnosed ovarian cysts.

Material and methods

Fetal ovarian cysts were diagnosed in 15 cases in our ultrasound unit during the period between Nov. 1, 1987, and March 31, 1990. Ten of these 15 cases were diagnosed during routine scans, and five were referred on the basis of a nonspecific diagnosis (made elsewhere) of a fetal abdominal mass.

These criteria were used in identifying ovarian cysts: (1) presence of a cystic structure that was regular in shape and usually located on one side of the fetal abdomen, (2) integrity of urinary and gastrointestinal tracts, and (3) female sex of the fetus (Fig. 1). The diagnosis was always presumptive, since rare lesions such as mesenteric cysts, urachal cysts, or enteric duplications could not be ruled out with absolute certainty. A careful ultrasonographic survey of the entire fetal anatomy, including the heart, was performed in all cases. Attention was paid to cyst septation or bilaterality. Torsion was assumed if intracystic flocculation was observed, which was followed by sedimentation on the sloping part of the cyst. This sedimentation gives a characteristic liquid interface (Fig. 2).[7] The volume of the ovarian cysts was calculated with the formula for determining the volume of a sphere ($4/3\ \pi\ r^3$). The radius was calculated as (D1 + D2 + D3)/6. The patients were followed up by a multidisciplinary team with a specialty in fetal anomalies. This team included an obstetrician, a neonatologist, a pediatric surgeon, a social worker, and a geneticist. Postnatal follow-up was carried out in all cases.

Results

Of the 15 cases of fetal ovarian cysts that were diagnosed prenatally, nine did not show any ultrasono-

From the Ultrasound Unit, Division of Obstetrics and Gynecology,[a] and the Department of Pediatric Surgery,[b] Soroka University Hospital, Faculty of Health Sciences, Ben-Gurion University of the Negev.
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Reprint requests: I. Meizner, MD, Ultrasound Unit, Division of Obstetrics and Gynecology, Soroka Medical Center, P.O. Box 151, Beer-Sheva 84101, Israel.
6/1/26199

graphic evidence of torsion (Table I). Gestational age at the time of diagnosis in these cases ranged from 19 to 35 weeks of pregnancy. In four cases the cystic mass was bilateral (Fig. 3). The cysts were unilocular and were without internal echoes or fluid levels. The largest was 6 × 7 × 4 cm.

In the remaining six cases evidence of torsion was observed (Table II). Gestational age at the time of diagnosis ranged from 19 to 37 weeks of pregnancy. In these cases internal echoes and fluid levels were seen, both of which suggested torsion. All cases were unilateral, and one cyst (Case 10) was multilocular. The mean diameter of the cysts in this group was 5.41 ± 0.25 cm, as compared to that of the group without torsion, which was 4.33 ± 0.3 ($p < 0.01$).

Patients were delivered of infants spontaneously in 13 cases, cesarean sections were performed in 2 cases (one of fetal distress and the other of placenta previa). All newborn were in good condition with normal Apgar scores. Each newborn weighed >2500 gm. There were no additional anomalies. The ultrasonographic scans performed during the neonatal period were consistent with the diagnosis of fetal ovarian cyst in all 15 cases. Postnatal ultrasonographic findings of the nine cases with no evidence of torsion were confirmed postnatally (Table I). In seven of these nine cases the cysts involuted within 2 months of follow-up, and in the remaining two cases the cysts disappeared within 6 months. There was no absolute proof that the cysts were indeed of ovarian origin. However, a regressing cystic lesion in a female newborn makes this diagnosis highly probable.

In six cases ultrasonography performed immediately after delivery confirmed the impression of torsion in ovarian cysts (Table III). The diagnosis was confirmed during operation (Fig. 4). In four cases adnexectomy was performed, and in one case excision of the cyst was sufficient. One of the cysts (case 12) was found to be detached from its origin as the result of ischemia of the fetal adnexa, and thus was identified as a "wandering" cyst. Histologic examination revealed follicular cysts in three cases and necrotic ovarian cysts with no specific epithelial findings in the remaining three cases. In all cases there was no evidence of cystic lesions in the contralateral ovary.

Comment

Except for large cysts and those complicated by torsion or hemorrhage, fetal ovarian cysts and tumors have not been recognized until recently, when an increase in the use of obstetric ultrasonography has made possible the earlier and more frequent diagnosis of ovarian cyst.

In eight of our patients the antenatal diagnosis of an ovarian cyst was made before the 28th week of gestation. We could not find any reports in the literature

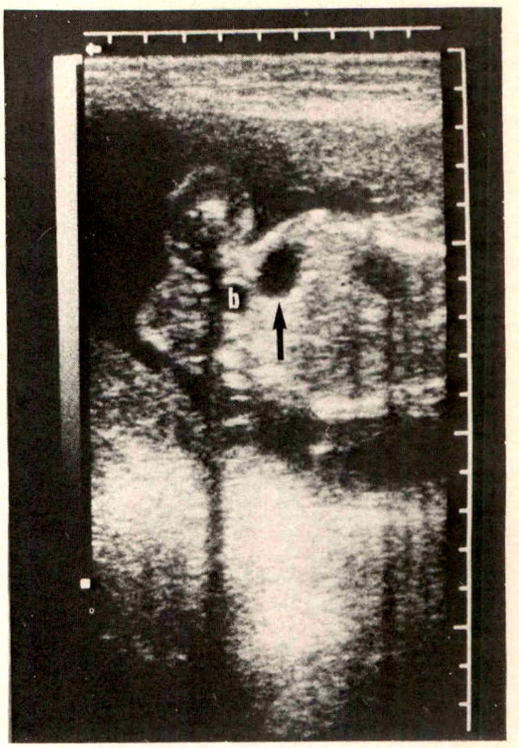


Fig. 1. Longitudinal scan of fetus at 20 weeks of gestation with small ovarian cyst *(arrow)*. *b*, Bladder.

that pertained to prenatal diagnosis of ovarian cysts before the third trimester of pregnancy.[5-9] Fetal ovarian cysts are the most common cause of an intraabdominal cyst that is observed antenatally (excluding those of renal and gastrointestinal etiology).[8] There is controversy with respect to the genesis of fetal ovarian cysts. Evidence suggests that excessive stimulation of the fetal ovary by both placental hormones and maternal hormones may be a significant factor in cyst development. Such stimulation would explain an increased incidence of cysts in the infants of mothers with diabetes, toxemia, or rhesus isoimmunization, presumably from excessive release of placental chorionic gonadotropins by the enlarged placenta.[10, 11] Suggested hypotheses include a precocious follicle-stimulating hormone peak between the 20th and 30th weeks of gestation, an abnormal human chorionic gonadotropin peak that would explain the observation of fetal ovarian cysts in pregnancies with increased secretion (perhaps from maternal diabetes or rhesus isoimmunization), or a disorder of enzyme activity in the internal theca. An association between the development of ovarian cysts and fetal hypothyroidism has also been suggested on the basis of nonspecific stimulation of pituitary glycoprotein hormone synthesis.[11, 12] None of the above hypotheses has been formally proved. In our present series there were no mothers with diabetes and the weight of each pla-

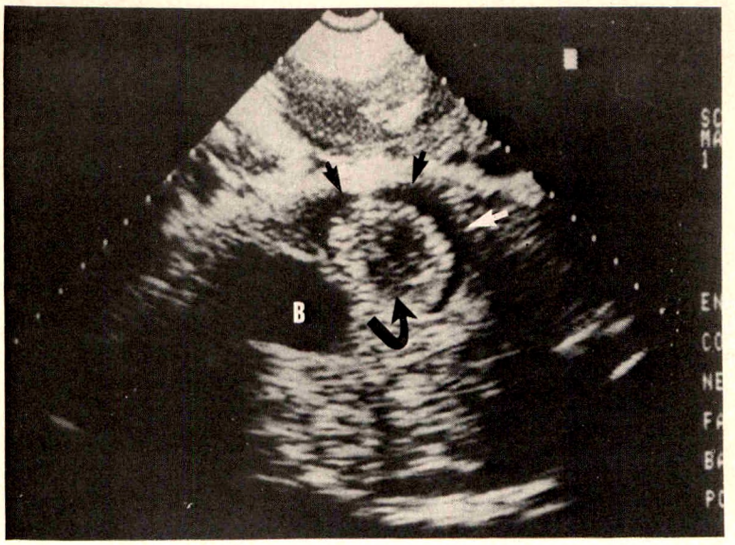


Fig. 2. Longitudinal scan of fetus at 32 weeks of pregnancy; torsion of ovarian cyst is indicated. *Small arrows* indicate cyst and *curved arrow* points to sediment deposited within its walls. *B*, Bladder.

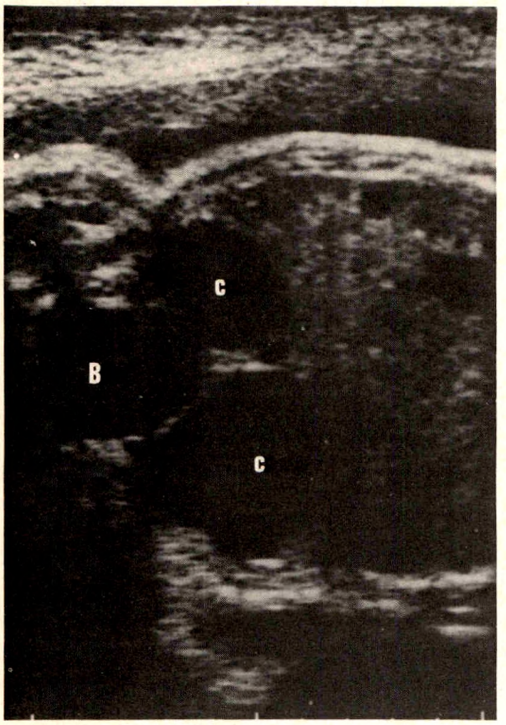


Fig. 3. Longitudinal scan of a fetus at 32 weeks of gestation with bilateral ovarian cysts. *C*, Cyst; *B*, bladder.



Fig. 4. Intraoperative finding of torsion of huge ovarian cyst.

centa ranged within the normal limit of one sixth to one fifth of each infant's respective birth weight. Sedin et al.[13] suggested that in premature babies estradiol-producing ovarian cysts may occur from inadequate negative feedback that results in ovarian hyperstimulation.

The ultrasonographic diagnosis of a rounded, intraabdominal liquid mass in a female fetus permits the longitudinal observation of these cysts. These cysts may either regress with no sequelae or remain stable. None of our cases showed antenatal regression. In one case a sudden development of intense hyperechogenicity

Table I. Prenatal and postnatal findings in nine cases with uncomplicated fetal ovarian cysts

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*	*Case 7*	*Case 8*	*Case 9*
Gestational age at diagnosis (wk)	19	32	21	29	35	20	21	19	24
Gestational age at delivery (wk)	41	39	41	38	39	40	40	36	37
Location and volume of ovarian cysts (cm^3)									
Antenatal ultrasonography	L 15.3	B 12.5	B 20.9	L 23.7	B 13.9	B 23.7	R 11.1	L 16.7	R 20.9
	—	20.9	18.1	—	25.0	18.1	—	—	—
Postnatal ultrasonography	L 23.7	B 11.1	B 25.0	L 23.7	B 16.7	B 27.8	R 13.9	L 15.3	R 20.9
	—	16.7	18.1	—	25.0	18.1	—	—	—
2 mo	L 8.3	None	L 11.1	None	None	None	None	None	None
6 mo	None	None	—	—	—	—	—	—	

All cysts were unilocular. Internal echoes or fluid levels were not observed in any of the cysts. *L*, Left; *R*, right; *B*, bilateral.

Table II. Prenatal and postnatal findings in six cases of suspected ovarian cysts with torsion

	Case 10	*Case 11*	*Case 12*	*Case 13*	*Case 14*	*Case 15*
Gestational age at diagnosis (wk)	32	37	30	28	19	20
Gestational age at delivery (wk)	39	41	40	40	38	40
Location and volume of ovarian cysts (cm^3)						
Antenatal ultrasonography	R 25	L 25	W 22.3	L 18.1	L 22.3	L 20.9
Postnatal ultrasonography	R 26.4	L 26.4	W 20.9	L 19.5	L 25	L 19.5

All cysts were unilateral. In case 10 the cyst was multilocular, and the remaining cysts were unilocular. In all cysts internal echoes and fluid levels were observed. *L*, Left; *R*, right; *W*, "wandering."

Table III. Intraoperative findings and management of six antenatally diagnosed complicated fetal ovarian cysts and their histologic features

Case	*Intraoperative findings*	*Surgery*	*Histologic type*
10	Right adnexa replaced by multilocular cyst	Right adnexectomy	Follicular cyst
11	Left cystic mass	Excision	Necrotic cyst
12	Right floating cystic mass	Removal	Necrotic cyst
13	Left adnexa replaced by cystic mass	Left adnexectomy	Follicular cyst
14	Left cyst with torsion	Left adnexectomy	Follicular cyst
15	Left cyst with torsion	Excision	Necrotic cyst

within the cyst, which was followed by a complex, heterogeneous appearance, was interpreted as the consequence of intrauterine torsion of the cyst with infarction. Torsion may be assumed when the presence of intracystic flocculation is followed by sediment that is deposited on the sloping part of the cyst, which gives a characteristic liquid interface.

Ultrasonography can accurately depict intracystic hemorrhage and necrosis. The ultrasonographic criteria for torsion were found to be reliable in all six cases and were confirmed at operation. Torsion may lead to necrosis of the pedicle and may thus cause a "wandering" tumor (case 3).[14]

All nine cysts without ultrasonographic signs of torsion regressed spontaneously during the first 6 months after delivery. This may be the general tendency of uncomplicated cysts. Therefore in these simple cysts ultrasonographic assessment during the first months of life should be the only measure taken. On the other hand, when ultrasonographic criteria indicate torsion, prompt surgical intervention immediately after delivery is the treatment of choice. Puncturing appears dangerous (frequent multiple pericystic adhesions may result) or impossible (where the content is of organized-coagulum type). The surgeon should try to preserve ovarian parenchyma whenever possible, but the state of adnexa and ovary often leaves no choice other than adnexectomy (Fig. 4).

The tendency of simple cysts to regress near term or in the early neonatal period does not justify in utero therapy and certainly does not necessitate induction of labor before lung maturity is ascertained. However, there are reports in the literature of ruptured cysts that required emergency laparotomy.[15] Therefore it was suggested that when a large ovarian cyst is suspected in a mature fetus, consideration should be given to a cesarean section to avoid the risks of torsion or rupture of the cyst.[16] Fetal ovaries that show ultrasonographic signs of torsion tend to have a poor outcome. If a simple fetal ovarian cyst begins to show signs of recent torsion

during follow-up and if the fetus is deemed viable, induction of labor may be considered to permit early surgical intervention in an effort to save an ovary.

In conclusion, fetal ovarian cysts can be diagnosed by ultrasonography early in pregnancy. Follow-up and assessment are possible throughout gestation. It seems to us that management depends on the antenatal ultrasonographic appearance of the cyst and on its evolution throughout pregnancy. Cysts without strict ultrasonographic evidence of torsion should be managed carefully after delivery. However, prenatal ultrasonographic evidence of torsion confirmed postnatally is an indication for prompt postnatal intervention.

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A comparison of flurbiprofen, tranexamic acid, and a levonorgestrel-releasing intrauterine contraceptive device in the treatment of idiopathic menorrhagia

Ian Milsom, MD, Kerstin Andersson, MD, Björn Andersch, MD, and Göran Rybo, MD
Göteborg, Sweden

Treatment with flurbiprofen (100 mg twice a day for 5 days), tranexamic acid (1.5 gm three times a day for 3 days and 1 gm twice a day for another 2 days), and an intrauterine contraceptive device releasing 20 μg levonorgestrel per day was compared in women with idiopathic menorrhagia. The menstrual blood loss during two control periods in 15 women subsequently treated with flurbiprofen and tranexamic acid was 295 ± 52 ml and 203 ± 25.2 ml in the 16 women later fitted with a levonorgestrel-releasing intrauterine contraceptive device. Menstrual blood loss was reduced by all three forms of treatment. The reduction in menstrual blood loss expressed as a percentage of the mean of two control cycles for each form of treatment was as follows: flurbiprofen, 20.7% ± 9.9%; tranexamic acid, 44.4% ± 8.3%; levonorgestrel-releasing intrauterine contraceptive device after 3 months, 81.6% ± 4.5%; levonorgestrel-releasing intrauterine contraceptive device after 6 months, 88.0% ± 3.1%; levonorgestrel-releasing intrauterine contraceptive device after 12 months, 95.8% ± 1.2%. The reduction in menstrual blood loss achieved by the levonorgestrel-releasing intrauterine contraceptive device was greater than that recorded with flurbiprofen ($p < 0.001$) and tranexamic acid ($p < 0.01$), and was greater for tranexamic acid when compared with flurbiprofen ($p < 0.05$). The levonorgestrel-releasing intrauterine contraceptive device was the only form of treatment to reduce mean menstrual blood loss below 80 ml per menstruation, the upper limit of normal menstrual blood loss. (Am J Obstet Gynecol 1991;164: 879-83.)

Key words: Menorrhagia, treatment, intrauterine contraceptive device, levonorgestrel, tranexamic acid, flurbiprofen

Approximately 10% of healthy women suffer from menorrhagia, defined as a menstrual blood loss of >80 ml.[1] Excessive menstrual blood loss may lead to iron-deficiency anemia and ultimately necessitate hysterectomy.[2, 3] Almost half a million hysterectomies are performed each year in the United States because of menstrual disorders[4] and it has been reported that >18,000 hysterectomies are performed in England each year solely because of menorrhagia.[5] Since operative treatment carries a not insignificant risk of morbidity and mortality, various forms of medical treatment have been advocated as the first line of treatment. Antifibrinolytic agents, prostaglandin synthetase inhibitors, oral contraceptives, and the intrauterine release of progestogens have all been shown to be effective in the treatment of idiopathic menorrhagia.[6-10]

The aim of this study was to objectively evaluate three principally different forms of medical treatment for idiopathic menorrhagia. The effect of treatment with flurbiprofen, a prostaglandin synthetase inhibitor; tranexamic acid, an antifibrinolytic agent; and intrauterine release of levonorgestrel by means of an intrauterine contraceptive device (IUD) were compared.

From the Department of Obstetrics and Gynecology, University of Göteborg, East Hospital.
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Reprint requests: Ian Milsom, MD, PhD, Department of Obstetrics and Gynecology, East Hospital, S-416 85 Göteborg, Sweden.
6/1/26281

Material and methods

Thirty-five women who sought medical attention because of heavy menstrual bleeding with a menstrual blood loss of >80 ml during two pretreatment periods were invited to participate in this study. Only patients judged to have idiopathic menorrhagia were included. The women had a normal-sized or only slightly enlarged uterus and no signs of pelvic pathologic conditions as judged by gynecologic examination. Patients with menorrhagia caused by pelvic pathologic conditions, e.g., palpable fibroids diagnosed by gynecologic examination, were excluded. The women were otherwise healthy, had regular menstrual cycles, had not used oral contraceptives or an IUD, and had not been pregnant during the 2 months before inclusion. The women gave informed consent and the study was approved by the Medical Ethics Committee, University of Göteborg.

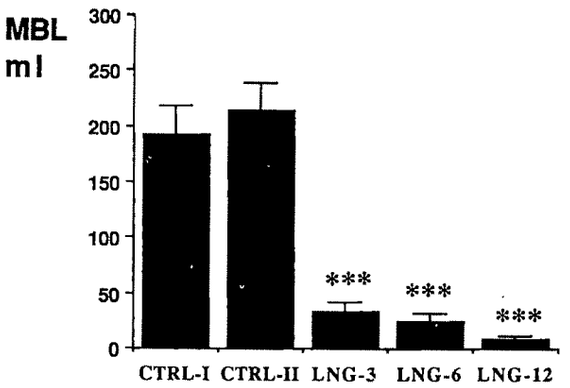


Fig. 1. Menstrual blood loss *(MBL)* in 16 women with idiopathic menorrhagia treated with a levonorgestrel-releasing IUD *(LNG)*. Menstrual blood loss was determined during two medication-free control cycles *(CTRL)* and 3, 6, and 12 months after insertion. Significance of difference between control cycles and during treatment with levonorgestrel-releasing IUD indicated by *triple asterisks* ($p < 0.001$).

The first twenty consecutive women (age, 37.8 ± 0.9 years; range, 31 to 45 years; and parity, 2.2 ± 0.2, range 1 to 4) were treated by the intrauterine release of levonorgestrel (LNG-IUD, Leiras, Turku, Finland). The levonorgestrel-releasing IUD used in this study has the same T-shaped polyethylene skeleton as the Nova-T IUD. The steroid is homogeneously dispersed in a polydimethyl-siloxane reservoir covered by a rate-limiting membrane on the vertical arm of the device. The total amount of levonorgestrel in the IUD is 46 mg and the release rate is 20 μg/day. This IUD has an estimated effective life span of 5 years. Measurement of menstrual blood loss was performed during two consecutive periods before IUD insertion and 3, 6, and 12 months after insertion.

Fifteen women (age, 40.5 ± 1.1 years, range 34 to 49 years; and parity 1.7 ± 0.2, range 0 to 3) were then treated with flurbiprofen and tranexamic acid during four consecutive menstrual periods according to a randomized, open-crossover study design. Measurements of menstrual blood loss during two control cycles were first performed. Menstrual blood loss was also determined during the subsequent four cycles during which the women received either oral tranexamic acid (1.5 gm three times daily for 3 days and 1 gm twice daily for another 4 days) or flurbiprofen (100 mg twice daily for 5 days). Treatment was started on the first day of menstruation; each patient received one compound for two menstrual periods and then the other for a further two menstrual periods. Medication was supplied in individual patient packs and compliance was checked by counting the remaining tablets after each course of treatment.

All women were requested to record details of the menstrual cycle (dates of last menstrual period, duration of menstruation, possible intermenstrual bleeding) and any side effects. No additional medication was allowed and patients did not receive iron supplementation during the study. The venous concentration of hemoglobin was measured each month after menstruation.

The women were supplied with polyethylene bags for each menstrual period in which the used sanitary pads and tampons were collected daily. The bags were delivered to the laboratory after the period. Menstrual blood loss was determined by spectrophotometric analysis of alkaline hematin as described by Hallberg and Nilsson[11] and modified by Newton et al.[12]

Statistical analysis. The results are expressed as mean ± SEM. A linear nonparametric test and Fisher's least significant difference test were used to evaluate statistical significance ($p < 0.05$ was considered significant).

Results

Sixteen of the 20 women fitted with a levonorgestrel-releasing IUD completed the 12-month observation period. One woman was excluded from the study because of expulsion of the IUD during the first menstrual cycle after insertion. She was 41 years old, para 3, and menstrual blood loss was 550 and 449 ml before insertion. A hysterectomy was later performed. The endometrium was normal at histopathologic examination, apart from the presence of small submucous fibroids. In two women the IUD was removed before the 6-month follow-up because of intermenstrual bleeding. One woman had the levonorgestrel-releasing IUD removed after 10 months because of acne, weight gain (2.5 kg), mood changes, and intermenstrual bleeding.

Menstrual blood loss during the two control periods for the women who subsequently completed the study was 203 ± 25.2 ml (range, 80 to 381 ml), decreasing ($p < 0.001$) to 34 ± 8.5 ml (range, 0 to 145 ml) after 3 months, 25 ± 7.3 ml (range, 0 to 119 ml) after 6 months, and 9 ± 2.7 ml (0 to 33 ml) after 12 months (Fig. 1). Intermenstrual bleeding or spotting was reported by most women during the first three cycles after insertion, but the frequency diminished gradually. The amount of blood loss by spotting was estimated by the women to be very small and was not measured. Seven women subsequently developed amenorrhea.

Menstrual blood loss during the medication-free menstrual cycles was 295 ± 52 ml (range, 81 to 701 ml) in the women subsequently treated with flurbiprofen and tranexamic acid (Fig. 2). The difference in menstrual blood loss before treatment between women later fitted with a levonorgestrel-releasing IUD and women treated with flurbiprofen and tranexamic acid was not statistically significant. Treatment with flurbi-

profen reduced ($p < 0.05$) mean blood loss to 223 ± 44 ml (range, 50 to 636 ml). The mean blood loss was reduced ($p < 0.01$) to 155 ± 33 ml (range, 36 to 511 ml) during treatment with tranexamic acid. Treatment with tranexamic acid or flurbiprofen did not alter the duration of menstrual flow and there were no reported episodes of intermenstrual bleeding. Seven of 15 women complained of side effects which they attributed to the given medication during treatment with tranexamic acid. Four of the 15 women complained of side effects during the flurbiprofen cycles. Treatment with tranexamic acid caused nausea, dizziness, numbness, "restless legs," headache, and, in three women, vomiting and difficulty in swallowing. Flurbiprofen caused tiredness, stomach pains, and nausea. No patient discontinued therapy with either flurbiprofen or tranexamic acid because of side effects.

The reduction in menstrual blood loss expressed as a percentage of the mean of two control cycles for each form of treatment is shown in Fig. 3. Menstrual blood loss reduction was greater with the levonorgestrel-releasing IUD compared with flurbiprofen ($p < 0.001$) or tranexamic acid ($p < 0.01$) and with tranexamic acid compared with flurbiprofen ($p < 0.05$).

The hemoglobin concentration during the control cycles (127.4 ± 3.7 gm/L) did not differ significantly from the recorded hemoglobin concentration during treatment with flurbiprofen (127 ± 3.4 gm/L) and tranexamic acid (126 ± 3.0 gm/L). The hemoglobin concentration for women subsequently fitted with a levonorgestrel-releasing IUD was 127 ± 2.6 mg/L and increased to 138 ± 2.1 mg/L after 6 months ($p < 0.05$) and to 139 ± 3.2 mg/L after 12 months ($p < 0.01$).

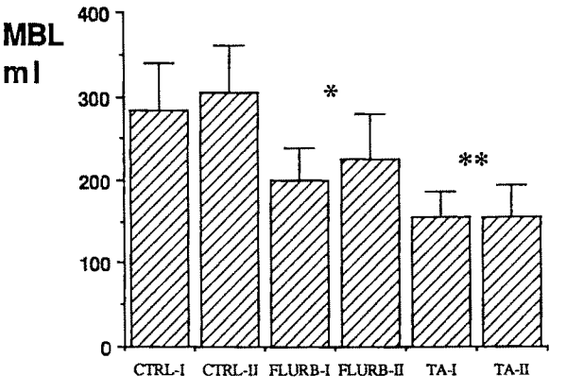


Fig. 2. Menstrual blood loss *(MBL)* in 15 women with idiopathic menorrhagia before and during treatment with flurbiprofen (100 mg twice daily for 5 days) and tranexamic acid (1.5 gm three times a day for 3 days and 1 gm twice daily for days 4 and 5). Significance of difference between control cycles *(CTRL)* and during treatment with flurbiprofen *(FLURB)* and tranexamic acid *(TA)* indicated by *asterisk* ($p < 0.05$) and *double asterisks* ($p < 0.01$).

Comment

In this study menstrual blood loss during two medication-free menstrual periods was well in excess of the clearly defined limit of 80 ml per menstrual period used in the definition of menorrhagia.[1] All patients included were deemed to be suffering from idiopathic menorrhagia by exclusion of gross pelvic pathologic conditions.

Increased fibrinolytic activity has been implicated as a cause of idiopathic menorrhagia.[13] Prostaglandins have also been shown to play an important role in the regulation of menstrual bleeding. Several reports have demonstrated an increased endometrial concentration of prostaglandins in women with dysfunctional bleeding and menorrhagia.[14, 15]

Antifibrinolytic agents are well established in the treatment of menorrhagia and have been shown to be highly effective in reducing excessive menstrual bleeding.[6] A variety of prostaglandin synthetase inhibitors have also been shown to be effective in the treatment of menorrhagia.[5, 16, 17] The doses of flurbiprofen and tranexamic acid used in this study are those presently used in clinical practice and have previously been shown to be optimal for the effective treatment of idiopathic menorrhagia.[6, 9, 17] Higher doses of tranexamic acid have been shown to reduce menstrual blood loss even more but are unfortunately associated with an increasing frequency of side effects such as nausea and gastric irritation. The use of intrauterine progesterone has been shown to be effective in the treatment of menorrhagia[8] and is associated with suppression of the endometrium caused by progesterone.[18]

In this study both flurbiprofen and tranexamic acid reduced menstrual blood loss in patients with idiopathic menorrhagia. However, neither substance reduced the mean menstrual blood loss to <80 ml. The reduction in menstrual blood loss was significantly greater during treatment with tranexamic acid compared with flurbiprofen. This may indicate that endometrial fibrinolysis, rather than prostaglandin release, is of greater importance as a cause of menorrhagia. Ylikorkala and Viinikka[17] compared antifibrinolytic and antiprostaglandin treatment in women with IUD-induced excessive menstrual blood loss. Treatment with tranexamic acid reduced menstrual blood loss by 56%, whereas the prostaglandin synthetase inhibitor diclofenac sodium reduced menstrual blood loss by 24%. Similar results were recorded in this study in patients with idiopathic menorrhagia. Tranexamic acid reduced menstrual blood loss by 44%, whereas treatment with the prostaglandin synthetase inhibitor flurbiprofen reduced menstrual blood loss by 21%.

The levonorgestrel-releasing IUD reduced menstrual blood loss to levels well below 80 ml. The

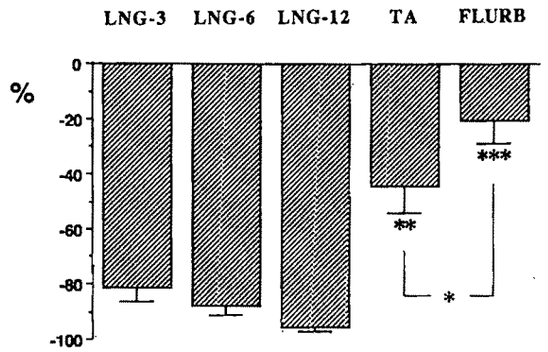


Fig. 3. Reduction in menstrual blood loss expressed as percentage of mean of two control cycles for each form of treatment. Significance of difference between treatment with levonorgestrel-releasing IUD *(LNG)* and tranexamic acid *(TA)* and flurbiprofen *(FLURB)* indicated by *double asterisks* ($p < 0.01$) and *triple asterisks* ($p < 0.001$) and between treatment with tranexamic acid and flurbiprofen indicated by *asterisk* ($p < 0.05$).

reduction in menstrual blood loss achieved by the levonorgestrel-releasing IUD was greater than that recorded during treatment with flurbiprofen and tranexamic acid. Levonorgestrel is not metabolized by the endometrium as rapidly as progesterone and has a more uniform and profound local effect.[19] The use of progesterone in an IUD (Progestasert) has been shown to decrease menstrual blood loss by 65% after 12 months of treatment in women with menorrhagia.[8] The levonorgestrel-releasing IUD used in the present study reduced menstrual blood loss by 82% after 3 months and by 96% after 12 months. It is known that the levonorgestrel-releasing IUD can cause amenorrhea in some women.[20] In seven of the women included in this study amenorrhea subsequently developed.

Conventional copper-bearing IUDs are a common form of birth control worldwide, but their use in the United States has been limited, mainly because of the reported risk of upper genital tract infections.[21] The use of the levonorgestrel-releasing IUD has been shown to be associated with a lower frequency of pelvic inflammatory disease than a conventional copper-bearing IUD.[20] A possible explanation for this observation is the local effect of the progestogen on the endometrium and cervical mucus, which provides protection against ascending infections. Further, long-term studies are at present being performed to compare the influence of the levonorgestrel-releasing IUD with copper IUDs regarding the risk of developing pelvic inflammatory disease.

There is no doubt that hysterectomy is a highly effective way of treating menorrhagia, but operative morbidity and long-term morbidity cannot be considered to be insignificant.[22] Can hysterectomy be justified if alternative, simpler treatment forms become available? Selective endometrial destruction with laser or electrocoagulation may in the near future provide a useful alternative to hysterectomy. However, there are at present few published reports of follow-up after endometrial resection.[23]

This study has demonstrated a reduction in menstrual blood loss by all three, principally different, medical treatment regimes under investigation. The levonorgestrel-releasing IUD reduced menstrual bleeding more than flurbiprofen and tranexamic acid, and was the only form of treatment to reduce menstrual blood loss to normal levels, i.e., <80 ml per menstrual period. The overall frequency of side effects with the levonorgestrel-releasing IUD was low. Our results strongly support the use of a levonorgestrel-releasing IUD as a useful alternative to hysterectomy for many women with idiopathic menorrhagia.

We wish to express our thanks to Mölnlycke AB for the kind donation of the pads used in this study.

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Position change and central hemodynamic profile during normal third-trimester pregnancy and post partum

Steven L. Clark, MD,[a, b] David B. Cotton, MD,[c] James M. Pivarnik, PhD,[c] Wesley Lee, MD,[c] Gary D.V. Hankins, MD,[d] Thomas J. Benedetti, MD,[e] and Jeffrey P. Phelan, MD[f]

Salt Lake City, Utah, Houston and San Antonio, Texas, Seattle, Washington, and Los Angeles, California

Central hemodynamic response to position change was assessed in 10 normotensive primiparous patients between 36 and 38 weeks' gestation. Studies were repeated between 11 and 13 weeks post partum. Compared with the left lateral position, we observed a mean 9% fall in cardiac output in the supine position and an 18% fall when patients were standing. When standing, these patients had a 30% increase in pulse and a 21% fall in left ventricular stroke work index. The orthostatic response after pregnancy was much more labile than that during the third trimester. These findings have important descriptive implications for the understanding of the human response to orthostasis during pregnancy, as well as clinical implications for patients at risk of uteroplacental insufficiency and for working women during pregnancy. (AM J OBSTET GYNECOL 1991;164:883-7.)

Key words: Hemodynamics of pregnancy, pulmonary artery catheter, critical care

Pregnancy brings about profound alterations in maternal hemodynamics.[1] The central hemodynamic profile of the pregnant woman is further altered by position changes, compounded in late pregnancy by the effects of the gravid uterus on vena caval and aortic blood flow in the supine position.[2] Whereas partial hemodynamic descriptions of select postural changes in pregnancy exist, these lack complete data with regard to preload of the left and right sides of the heart, pulmonary vascular pressures, and left ventricular function or have relied on noninvasive assessment of cardiac output.[2, 3] We present a systematic description of the effects of position change on maternal central hemodynamics with the use of the Fick principle to assess cardiac output and pulmonary artery catheterization to measure central pressures.

From the Intermountain Health Care Perinatal Centers,[a] The University of Utah,[b] Baylor University,[c] Wilford Hall United States Air Force Hospital,[d] The University of Washington,[e] and Queen of the Valley Hospital.[f]
Received for publication June 28, 1990; accepted November 9, 1990.
Reprint requests: Steven L. Clark, MD, Intermountain Health Care Perinatal Centers, LDS Hospital, 8th Ave. and "C" St., Salt Lake City, UT 84143.
6/1/26702

Material and methods

Ten volunteers were studied. Each met criteria detailed in Table I.

Written informed consent was obtained. The study was approved by the hospital Institutional Review Board and an outside reviewer (pulmonologist-intensivist). Subjects were reimbursed $200.00 for participation in each phase (pregnant and nonpregnant) of the study. Four subjects who volunteered were excluded before initiation of the study because of borderline anemia, fetal intrauterine growth retardation, or the development of elevated blood pressure before

Table I. Study inclusion criteria

Primiparous
Maternal age <26 years
Singleton vertex fetus
Gestational age between 36 and 38 weeks confirmed by ultrasonography
No fetal intrauterine growth retardation or anomalies
Normal amniotic fluid volume
Normal maternal weight for height
Normal maternal hematocrit values
No maternal alcohol, tobacco, or drug use
No maternal hypertension, medical illness, or medications

the study. Subjects were initially recruited from physicians' offices in Provo, Utah. The study was performed at the Utah Valley Regional Medical Center in Provo, altitude 4540 feet.

Each subject underwent pulmonary artery catheterization via the subclavian route and radial arterial line placement. Venous access was established by means of a peripheral heparin lock venous catheter. Baseline hemodynamic assessment was made with subjects in the left lateral recumbent position after a 30-minute stabilization period. A 10-minute premeasurement stabilization period was allowed between each position change. Cardiac output was measured with the use of the direct Fick technique. Oxygen consumption was measured continuously with the open circuit technique with a metabolic cart (Sensormedics model 4400, Anaheim, Calif.). Before each test, the metabolic analyzers were calibrated with certified standard gases of known concentrations verified by the micro-Scholander technique. When each subject's oxygen consumption values stabilized (after 10 minutes), blood samples were obtained simultaneously from the pulmonary and radial arteries. Care was taken to clear the lines before drawing blood to prevent dilution of samples. Blood samples were analyzed in duplicate for oxygen content on a blood gas analyzer (Corning Model 168, Medfield, Mass.). Calibrations were performed before each subject was tested and routinely throughout each experiment. Central pressures were measured and recorded with a 10-channel physiologic monitor-recorder (Hewlett Packard model 7785 B, Waltham, Mass.). Central pressures presented here represent the mean during three consecutive respiratory cycles as measured in end expiration from the tracing. Monitors were rezeroed in each position with transducer placement at the level of the left atrium confirmed by simultaneous real-time maternal echocardiography. Studies were repeated in each subject between 11 and 13 weeks post partum.

Results

Table II describes hemodynamic changes that occur as a result of position change in the pregnant woman. Table III describes such changes in the same women after pregnancy. In pregnant patients, we observed a mean 9% fall in cardiac output associated with assumption of the supine position ($p < 0.05$) and a mean 18% fall on assumption of the standing position ($p < 0.05$) compared with the left lateral position. There was no significant change in cardiac output in the right lateral or knee-chest positions compared with the left lateral position. A decrease in cardiac output was also seen in the sitting position; however, this did not reach statistical significance (Fig. 1). No change in heart rate was observed on assumption of the supine position in these subjects. However, a 30% increase in pulse, a 21% fall in left ventricular stroke work index, and a 54% rise in pulmonary vascular resistance was noted after standing ($p < 0.05$). No significant change was seen in mean systemic vascular resistance, mean arterial pressure (MAP), pulmonary capillary wedge pressure, central venous pressure, or mean pulmonary artery pressure with any position change, although a trend was observed toward a fall in central venous pressure with standing and a rise in central venous pressure with assumption of the knee-chest position.

After pregnancy, no change in any hemodynamic parameter was seen in the supine position, as compared with the left lateral position. A 31% fall in cardiac output, a 27% rise in heart rate, a 42% rise in systemic vascular resistance, a 106% rise in pulmonary vascular resistance, and a 22% fall in left ventricular stroke work index were observed when standing ($p < 0.05$). No significant change was seen in MAP, pulmonary capillary wedge pressure, central venous pressure, or mean pulmonary artery pressure.

Comment

The human response to orthostasis is complex. Major mediators of this response appear to be the carotid baroreceptors, which respond to decreased stretch by increasing adrenergic sympathetic output with resultant changes in heart rate and arterial resistance vessels.[4] These changes serve to maintain blood pressure despite an orthostatic fall in cardiac output. In contrast, venous capacitance vessels behave in a predominantly passive manner; the existence of venoconstriction in response to orthostasis is controversial and if present it is quantitatively of little significance.[4, 5] A fall in renal blood flow and glomerular filtration rate resulting in increased blood volume is also seen in response to orthostasis; similarly, blood volume decreases in the recumbent position.[4, 6, 7] Although they do not have an immediate hemodynamic effect, these renal excretory responses are extremely sensitive, and compared with the sluggish response in venous tone, imply that in human beings volume is adjusted preferentially to capacity.[4]

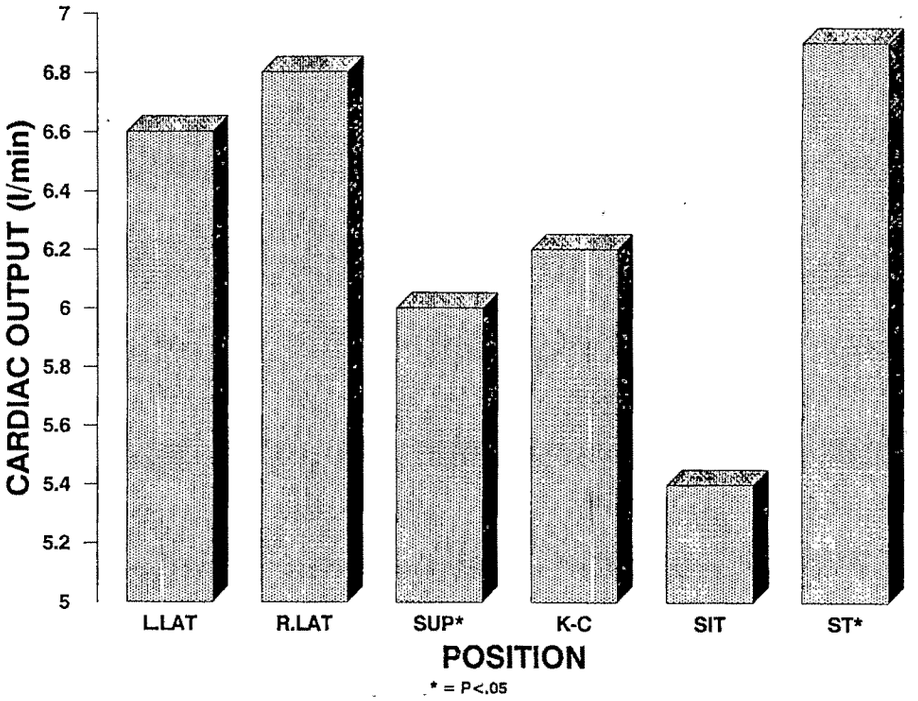


Fig. 1. Effect of position change on cardiac output during pregnancy. *L.LAT*, left lateral; *R.LAT*, right lateral; *SUP*, supine; *K-C*, knee-chest; *SIT*, sitting; *ST*, standing.

Table II. Hemodynamic alterations in response to position change late in third-trimester of pregnancy

	Position					
Hemodynamic parameter	*LL*	*RL*	*SUP*	*SIT*	*ST*	*KC*
MAP (mm Hg)	90 ± 6	90 ± 5	90 ± 8	90 ± 8	91 ± 14	95 ± 6
CO (L/min)	6.6 ± 1.4	6.8 ± 1.3	6.0 ± 1.4*	6.2 ± 2.0	5.4 ± 2.0*	6.9 ± 2.1
P (beats/min)	82 ± 10	87 ± 12	84 ± 10	91 ± 11	107 ± 17*	93 ± 12
SVR (dyne · cm · sec^{-5})	1210 ± 266	1321 ± 305	1437 ± 338	1217 ± 254	1319 ± 394	1223 ± 289
PVR (dyne · cm · sec^{-5})	76 ± 16	81 ± 33	101 ± 45	102 ± 35	117 ± 35*	79 ± 27
PCWP (mm Hg)	8 ± 2	7 ± 1	6 ± 3	4 ± 4	4 ± 2	8 ± 3
CVP (mm Hg)	4 ± 3	3 ± 2	3 ± 2	1 ± 1	1 ± 2	3 ± 2
LVSWI (gm · min · m^{-2})	43 ± 9	42 ± 9	40 ± 9	44 ± 5	34 ± 7*	47 ± 9

CO, Cardiac output; *P*, pulse; *SVR*, systemic vascular resistance; *PVR*, pulmonary vascular resistance; *PCWP*, pulmonary capillary wedge pressure; *CVP*, central venous pressure; *LVSWI*, left ventricular stroke work index; *LL*, left lateral; *RL*, right lateral; *SUP*, supine; *SIT*, sitting; *ST*, standing; *KC*, knee-chest.

*$p < 0.05$, Compared with left lateral position.

Our data indicate that during pregnancy, hemodynamic mechanisms are altered significantly. The major factors involved are decreased systemic vascular resistance and expansion of venous capacitance of the developing uteroplacental vascular bed and a 40% to 50% increase in intravascular volume and cardiac output.[1, 8] We observed a significant fall in cardiac output in response to orthostasis during pregnancy; the 30% increase in pulse was insufficient to compensate for declining stroke volume. These findings are similar in magnitude to those observed by Easterling et al.[3] with the use of Doppler cardiac output assessment. The fall in left ventricular stroke work index suggests a concomitant decrease in intrinsic ventricular function during orthostasis, almost certainly a result of Starling forces. However, these changes were far less than the orthostatic changes observed in the same patients while not pregnant (Fig. 2). Although MAP and mean pulmonary artery pressure were maintained during orthostasis after pregnancy, this was at the expense of

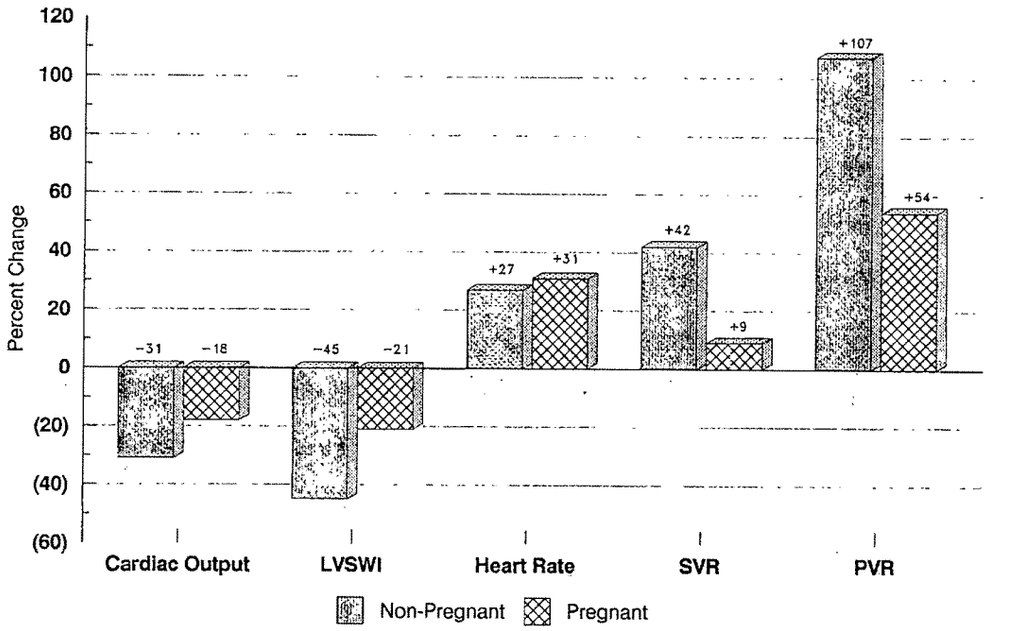


Fig. 2. Position change and hemodynamic parameters during pregnancy and post partum. *LVSWI*, Left ventricular stroke work index; *SVR*, systemic vascular resistance; *PVR*, pulmonary vascular resistance.

Table III. Hemodynamic alterations in response to position change in nonpregnant women

Hemodynamic parameter	*Position*		
	LL	*SUP*	*ST*
MAP (mm Hg)	86 ± 8	85 ± 9	79 ± 13
CO (L/min)	4.2 ± .6	4.7 ± 1.0	2.9 ± .6*
P (beats/min)	71 ± 10	70 ± 14	90 ± 19*
SVR (dyne · cm · sec^{-5})	1530 ± 520	1598 ± 568	2175 ± 709*
PVR (dyne · cm · sec^{-5})	123 ± 38	140 ± 87	254 ± 167*
PCWP (mm Hg)	6 ± 2	8 ± 3	3 ± 2
CVP (mm Hg)	4 ± 3	4 ± 3	1 ± 2
LVSWI (gm · min · m^{-2})	42 ± 7	39 ± 12	23 ± 11*

CO, Cardiac output; *P*, pulse; *SVR*, systemic vascular resistance; *PVR*, pulmonary vascular resistance; *PCWP*, pulmonary capillary wedge pressure; *CVP*, central venous pressure; *LVSWI*, left ventricular stroke work index; *LL*, left lateral; *SUP*, supine; *ST*, standing.

*$p < 0.05$, Compared with left lateral position.

dramatic increases in systemic vascular resistance (42%) and pulmonary vascular resistance (106%), changes of a degree not seen during pregnancy. Thus the stabilizing effects of increased intravascular volume during pregnancy are relatively greater than the somewhat opposite effects of increased venous capacitance and decreased systemic vascular resistance. This implies a greater hemodynamic stability in response to orthostasis during pregnancy despite a reported blunting of norepinephrine secretion in response to orthostatic stress.[9, 10] In nonpregnant human beings, response to orthostatic stress is similarly enhanced by increasing blood volume.[4] Such a condition would have important effects on the maintenance of constant uteroplacental blood flow because orthostasis, as opposed to recumbency, is in fact the dominant body position during pregnancy.

It was surprising that cardiac output during the late phase of pregnancy was actually lower in the standing position than that in the supine position. Whereas avoidance of the supine position has long been a mainstay of the management of the late phase of pregnancy, these results have significant negative implications in terms of cardiac output and uteroplacental perfusion for working women who must stand motionless for long periods of time (for example a bank teller or cashier). This would especially apply to women who otherwise are at risk of uteroplacental insufficiency. These results

give a physiologic basis for findings by Naeye et al. of decreased birth weight and placental infarction in working mothers who stand.[11] Potentially harmful effects of standing on decreased cardiac output would likely be negated by ambulation or exercise.

In the supine position, cardiac output falls significantly during the late phase of pregnancy because of vena caval and aortic compression by the pregnant uterus and resultant decreased venous return. The actual change is partially dependent on the degree of paravertebral collateral circulation that is available for returning blood from the lower extremities and pelvis to the heart. Our results with the Fick cardiac output assessment confirmed data obtained with noninvasive techniques.[3] Because the vena cava is located on the right of the descending aorta, the left lateral position has often been assumed to be the position of optimal cardiac output during the late phase of pregnancy. Whereas we confirmed the potentially deleterious effects of the supine position on cardiac output, we were unable to show any benefit (in terms of cardiac output) of left versus right lateral recumbent positions.

It is of interest that the rise in pulmonary vascular resistance necessary to maintain mean pulmonary artery pressure in the face of falling cardiac output was proportionately much greater than the rise in systemic vascular resistance necessary to maintain MAP with the same decrease in cardiac output. This was observed both during and after pregnancy. During pregnancy, we observed a 54% increase in pulmonary vascular resistance compared with a 9% rise in systemic vascular resistance with orthostasis, with no change in mean pulmonary artery pressure or MAP. After pregnancy, orthostasis resulted in a 106% rise in pulmonary vascular resistance and a 42% rise in systemic vascular resistance with no change in pulmonary or systemic pressures. These changes highlight the extremely labile nature of pulmonary vascular resistance compared with systemic vascular resistance during pregnancy. Such a feature is essential given the physiologic imperative to avoid on a minute-to-minute basis, ventilation-perfusion mismatch, and to effect appropriate recruitment of nonperfused vessels in the upper lungs, according to an ever-changing physiologic milieu. Such a labile system would be especially vital during a period of profound hemodynamic change, such as pregnancy, labor, and delivery. This may help explain why women with fixed pulmonary vascular resistance and pulmonary hypertension often tolerate so poorly the hemodynamic fluctuations associated with pregnancy and delivery.[12]

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Nonsurgical management of tubal pregnancy

Necessity in view of the changing clinical appearance

M. Pansky, MD, A. Golan, MD, I. Bukovsky, MD, and E. Caspi, MD
Tel Aviv, Israel

The incidence of ectopic pregnancy is definitely increasing. Ectopic pregnancies are diagnosed earlier these days because of the improvement in diagnostic means and the increasing awareness of the condition. It seems that there is a dramatic change in the clinical presentation of this disease; it used to be a grave and life-threatening condition, and now it is a more benign presentation. The vast majority of the diagnosed ectopic pregnancies are unruptured. This has stimulated various investigators to attempt nonsurgical methods of treatment such as systemic administration of methotrexate or RU 486 (mifepristone) or local injection of methotrexate, potassium chloride, or prostaglandins under laparoscopic or ultrasonographic guidance. Most of these conservative, nonsurgical measures proved efficient in 80% to 90% of cases. Expectant management, which was practiced in some cases, proved to be equally successful. We believe that the ideal mode of treatment in early unruptured ectopic pregnancy is still to be determined. The answer probably lies in proper selection. (Am J Obstet Gynecol 1991;164:888-95.)

Key words: Nonsurgical management, tubal pregnancy

The incidence of tubal pregnancy has undoubtedly been increasing and has even been described as an epidemic.[1] During the past 10 years, the incidence of ectopic pregnancy increased in our medical center from 1 of every 136 deliveries in 1979 to 1 of every 79 in 1989.

Some recent developments may explain this increasing incidence. First, the rising incidence of pelvic inflammatory disease, liberal use of tuboplastic and conservative operations for tubal pregnancy, and an increased use of agents to induct ovulation and in vitro fertilization–embryo transfer techniques[2]; second, there has been a dramatic improvement in the means of diagnosis, such as laparoscopy, sensitive β human chorionic gonadotropin (β-hCG) assays in urine and blood, and improvements in ultrasonographic techniques—mainly the introduction of transvaginal ultrasonography.[3,4] All these factors have dramatically changed the clinical picture of tubal pregnancy from a drastic and life-threatening disease to a more benign and less dangerous condition. In a series of 191 ectopic pregnancies that were diagnosed in our department during 1987 to 1989, 98% of patients were admitted because of clinically mild cases that needed no immediate emergency surgery. All had serum β-hCG determination before diagnosis and in 98% of patients at least one ultrasonographic examination was performed before diagnosis was established by laparoscopy or laparotomy. A ruptured tube was found in only 20% and active bleeding was found in only 10% of the patients (unpublished data).

It seems that the very early diagnosis of such pregnancies is also responsible, in part, for the increasing incidence because many of these early ectopic gestations remained undiagnosed in the past and were probably reabsorbed.

The dramatic change in the clinical picture of the disease and the drive to adjust the management to the new form of the disease, which is especially oriented to decrease the need for laparotomy and, as a direct result, to minimize the postoperative morbidity and recovery time, have recently stimulated many investigators to design various new and nonsurgical methods of treatment for early unruptured tubal pregnancies.[5]

The aim of the present analysis of these new modes of treatment is to reach some conclusions as to the indications, success rates, morbidity, and subsequent tubal patency of the various nonsurgical methods.

From the Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, affiliated with Sackler School of Medicine, Tel Aviv University.
Reprint requests: M. Pansky, MD, Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, Zerifin, Israel 70300.
6/1/24759

Table I. Results of systemic treatment with methotrexate for tubal pregnancy

Author	No. of cases treated	Failure rate		Dose of methotrexate	Route of administration	CV factor	Tubal patency	
		No.	%				No.	%
Miyazaki et al.[10]	9	1	12	60-300 mg	IM	No	5/6	83
Ory et al.[11]	6	1	17	1 mg/kg × 4 days	IV	Yes	—	—
Goldstein et al.[12]	13	1	8	1 mg/kg × 4 days 200 mg/m²	IM IV	Yes	4/4	100
Rodi et al.[13]	7	0	0	1 mg/kg × 4 days	IM	Yes	4/5	80
Sauer et al.[14]	21	1	5	1 mg/kg × 4 days	IM	Yes	15/20	75
Hans et al.[15]	1	0	0	50 mg × 5 days	IM	Yes	—	—
Ichinoe et al.[16]	22	1	4.5	0.4 mg/kg × 5 days	IM	No	10/19	53
Stovall et al.[17]	36	2	5.5	1 mg/kg × 1-4 days	IM	Yes	—	—
Chotiner et al.[18]	1	0	0	25 mg/kg × 3 days	IM	Yes	—	—
Brandes et al.[19]	1	0	0	1 mg/kg × 3 days	IM	Yes	1/1	100
Cowan et al.[20]	1	0	0	1 mg/kg × 3 days	IM	Yes	—	—
TOTAL	118	7	6				39/55	71

CV, Citrovorum; *MTX*, methotrexate; *IM*, intramuscular; *IV*, intravenous.

Medical treatment

Certain drugs are capable of acting on the trophoblastic cells and can arrest their growth and thus stop the development of the ectopic pregnancy, which is consequently absorbed. These can be introduced systematically or locally. Surgery can be avoided, and thus postoperative morbidity is minimized and recovery time is shortened. Moreover, the minimal surgical intervention, if any, in the pelvis minimizes the chance of adhesion formation and thus improves the future fertility rate.

Systemic administration

Methotrexate. Methotrexate is the prototype of the medical treatment of ectopic pregnancy, mostly because of its efficient action in stopping the development of the trophoblast. The drug inhibits the action of dihydrofolate reductase, which participates in the process of conversion of dihydrofolic acid to tetrahydrofolic acid. This process is essential to the synthesis of timin and purin, which take part in the synthesis of deoxyribonucleic acid (DNA). Methotrexate was introduced in 1956[6] and was the principal drug that was used in the treatment of gestational trophoblastic disease.

The first use of methotrexate in ectopic pregnancy was reported by Lathrop and Bowles[7] in 1963; they treated an abdominal pregnancy systemically with methotrexate and avoided a complicated operation.

Tanaka et al. in 1982[8] used methotrexate successfully in a patient with an interstitial pregnancy and demonstrated normal tubal patency afterward. A year later, the first attempt at treatment of a cervical pregnancy was reported by Farabow et al.[9] Although eventual surgery was not prevented, a definite decrease in pregnancy volume was reported, which probably ensured safer surgery.

The first series, although small, of ectopic pregnancies that were treated with systemic methotrexate was reported in the Japanese literature by Miatzaki et al.[10] in 1983. In eight patients surgery could be avoided. The author emphasizes the fact that the concentration of β-hCG at the time of admission is one of the major determinants of success.

Ory et al. in 1986[11] were the first to report in Western literature on six cases of unruptured tubal pregnancy that were treated with systemic methotrexate. In his small series, one patient required a laparotomy for intraperitoneal hemorrhage, and two others received blood transfusions. Of all the reported tubal pregnancies that were treated until now with systematic methotrexate, we found 118 such cases,[12-20] of which there were seven failures that required surgery—an incidence of 5.9% (Table I).

Of these seven failures, in two cases no details were given concerning pregnancy size, notice of fetal pulse, or β-hCG level at the time of admission.[10, 16] In the other five cases, β-hCG level was above 5000 mIU/ml and in four cases, fetal pulse was recorded by ultrasonography. In one patient active fimbrial bleeding was also reported.[12]

It seems that the key to the selection of suitable cases to ensure the maximum success rate is the establishment of solid criteria. In a review of the reports published until now, we found the following criteria to be important: (1) pregnancy diameter of <3 cm,[12-14, 17] (2) unruptured tube,[10-17] (3) no active bleeding,[10, 11, 13, 14, 17] (4) β-hCG serum level <1500 mIU/ml,[12] (5) no fetal pulse during ultrasonography,[12] and (6) less than 100 ml of blood in the pouch of Douglas.[11] The first three criteria define the early unruptured tubal pregnancy, which is mandatory for any nonsurgical treatment. The level of β-hCG and the identification of fetal heart

Table II. Side effects after systemic methotrexate therapy

Author	No. of cases treated	Cases with side effects		Dermatitis	Stomatitis	Gastritis	LFT	BMS	Pleuritis
		No.	%						
Miyazaki et al.[10]	9	3	33	—	3	—	3	—	—
Ory et al.[11]	6	3	50	—	2	1	2	—	—
Goldstein et al.[12]	13	0	0	—	—	—	—	—	—
Rodi et al.[13]	7	2	28	—	2	—	2	—	—
Sauer et al.[14]	21	5	23	1	2	1	1	—	1
Hans et al.[15]	1	0	0	—	—	—	—	—	—
Ichinoe et al.[16]	22	5	22.5	—	—	—	4	1	—
Stovall et al.[17]	36	3	8.3	—	1	—	2	—	—
Chotiner et al.[18]	1	1	100	—	—	1	—	1	—
Brandes et al.[19]	1	0	0	—	—	—	—	—	—
Cowan et al.[20]	1	0	0	—	—	—	—	—	—
TOTAL	118	22	18.6	—	10	2	14	2	1

LFT, Liver function tests; *BMS*, bone marrow suppression.

beat by ultrasonography are additional necessary safeguards. The adoption of these criteria for selection is reducing the number of cases that are suitable for this type of management to only 12% to 32% of all ectopic pregnancies.[11, 17]

Methotrexate unfortunately has several side effects, which in rare instances can also be fatal.[21] In the majority of cases the side effects are dose-dependent. Gastrointestinal side effects, which are the most common, include stomatitis and diarrhea. Other side effects, such as bone marrow suppression, impaired liver function, dermatitis, and pleuritis were also reported. The total incidence of side effects among the patients who were treated with methotrexate for ectopic pregnancy is reported as 20% (Table II).

An interesting attempt to reduce the rate of side effects was made by Stovall et al.[17] who tried to individualize the dose of methotrexate to the dynamic decrease in β-hCG levels by reducing the daily dose of methotrexate, according to change in the β-hCG levels. With this method, only 20% of the patients received four doses of methotrexate (1 mg/kg/day), compared with most of the other protocols, which treated all the patients with four to five doses of methotrexate. Stovall et al.[17] succeeded in reducing the rate of side effects to 8.3% without changing the success rate (Table II). Other investigators tried to overcome the problem of side effects by concurrent administration of citrovorum factor.[11-15, 17-20]

The future patency of the affected tube and the eventual fertility is in fact the ultimate parameter with which to evaluate the actual effectiveness of each mode of therapy. This is not an easy task because the numbers of cases and series are small. However, most researchers who examined the subsequent tubal patency after the systematic use of methotrexate reported an incidence of 53% to 100%, with an average incidence of 71% (Table I). This figure compares quite satisfactorily with the incidence of patency for various surgical methods.[22]

Actinomycin D. Actinomycin D acts as a ribonucleic acid polymerase inhibitor by binding with double helical DNA and blocking the transcription of the DNA molecule. Actinomycin D is known to have less hepatic and renal toxicity than methotrexate.

The first and only successful case of systemic treatment with actinomycin D was reported by Altaras et al.[23] They treated a patient who was shown to have an interstitial pregnancy with 0.5 mg/day actinomycin D (intraveneously) for 5 days and demonstrated an immediate pattern of decreasing β-hCG levels and bilateral tubal patency several weeks later.

RU 486 (mifepristone). The introduction of RU 486 (mifepristone) as a chemical abortifacient for intrauterine pregnancies[24, 25] has led some researchers to the conclusion that it may also act on ectopic pregnancies. RU 486 is an antagonist for the synthesis of progesterone in the corpus luteum and by this means arrests trophoblastic development.[24, 25] The first case that was treated was accidental,[26] in the treatment of what was thought to be an intrauterine pregnancy, which was later found to be a tubal pregnancy that showed no response to treatment. Kenigsberg et al.[27] treated residual tubal pregnancy with 650 mg of RU 486 and no decreases in serum β-hCG level and progesterone were shown. This pregnancy was later terminated with systemic methotrexate treatment.

Local injection

In view of the high toxicity and the potential side effects of the drugs that are given systematically, investigators were searching for a direct way to introduce a small amount of the drug to the pregnancy site. Technical improvement of laparoscopy and transvaginal ultrasonographic techniques enabled some investigators

to administer local injections into the ectopic site and thus drastically reduce the dose of the drug required and avoid the potential side effects and the need for surgery.

Transvaginal ultrasonography

METHOTREXATE. Feichtinger and Kemeter[28] in 1987 were the first to use transvaginal ultrasonography for suction and injection of methotrexate into the tubal gestational sac of a patient who had undergone in vitro fertilization. Only 10 mg of methotrexate was used, which is about 5% of the usual systemic dose. The β-hCG level at the time of injection was 500 mIU/ml, and no fetal pulse was detected. With the same protocol, the same researchers recently reported[29] eight more cases that were treated by transvaginal ultrasonographic local injection of methotrexate and showed a success rate of 89%.

Robertson et al.[30] in 1987 reported a less successful experience with suction and injection of methotrexate with the use of transvaginal ultrasonography. They had to operate on five of nine patients who were previously treated by this method because of continuing development of adnexal masses and rising β-hCG levels.

Leeton and Davison[31] reported on their successful experience in the treatment of two cases of tubal pregnancy that were diagnosed early, before any symptoms appeared, in patients who had undergone in vitro fertilization. Fetal heart beat was demonstrated in the tube in both cases. Suction and transvaginal injection of 50 mg of methotrexate into the gestational site was performed and showed complete resolution of the tubal pregnancies.

POTASSIUM CHLORIDE. Robertson et al.[32] tried to avoid surgery in a complicated case of heterotopic pregnancy in a patient who had undergone in vitro fertilization, and they chose not to use methotrexate because of a concomitant intrauterine pregnancy. The locally injected 20% solution of potassium chloride was supposed to induce cardiac arrest of the ectopic fetus and thus cause absorption of the ectopic pregnancy. The injection was performed transabdominally, under ultrasonographic guidance, and was anticipated by suction of the content of the sac. This attempt proved successful, the ectopic pregnancy was terminated and absorbed, and the intrauterine pregnancy was saved. They treated two more patients with the same method, both of whom required a later laparotomy for rising β-hCG levels and development of an adnexal mass.

Timor-Tritsch et al.[33] in 1988 reported another successful case of vaginal injection of potassium chloride into a tubal pregnancy in which a fetal pulse was detected, and the absorption of the pregnancy content was achieved.

The main advantage of the use of potassium chloride as opposed to methotrexate is its relative lack of systemic side effects. Its main drawback, which may be responsible for failure in some cases, is that potassium chloride acts mainly on the fetal heart and causes asystole and fetal death. This drug has no effect on the trophoblastic tissue, which may continue to grow even without a developing fetus and thus induce rupture of the tube.

PROSTAGLANDINS. Ribic-Paucelj et al.[34] recently reported on their preliminary experience with ultrasonography for local injection of prostaglandin E_2 (PGE_2) for unruptured tubal pregnancy. Two patients were treated with this method; no effect on β-hCG levels was shown, and the patients required surgery later. Feichtinger and Kemeter,[29] in the same year, reported on a single case that was treated with transvaginal ultrasonographic local injection of PGE_2. Again no effect was shown in the β-hCG level, and laparotomy had to be performed to terminate the pregnancy. In addition, this patient experienced severe gastrointestinal discomfort, such as cramps and vomiting.

Analysis of these results seems to show that local injection of PGE_2 with the use of ultrasonography is less efficient in comparison with other drugs that are used in the same way.

Usually transvaginal ultrasonographic injection was exercised in patients who had undergone in vitro fertilization or in other patients who were at high risk of the development of ectopic pregnancy.[28, 31, 32] In the vast majority of the unruptured ectopic pregnancies, the diagnosis is not finally established by ultrasonography, and therefore laparoscopy is indispensable. In our series of 191 ectopic pregnancies, 70% underwent at least one transvaginal ultrasonographic examination, and ectopic pregnancy was definitely diagnosed by ultrasonography in only 7% (unpublished data).

A question still to be answered is whether the suction of the contents of the gestational sac or the injection of methotrexate, potassium chloride, or PGE_2 is the direct cause of success in these cases. A controlled, randomized study in which suction only or injection only is performed is needed to clear this dilemma.

Laparoscopy. The laparoscope is considered to be the principal means of diagnosis of ectopic pregnancy. In a recent study that was performed in our department and included 233 patients with suspected ectopic pregnancies, 198 (85%) underwent diagnostic laparoscopy with a 99% success rate in the diagnosis or ruling out of ectopic pregnancy.

The ability to diagnose most ectopic pregnancies and to treat them during the same procedure is an important advantage of laparoscopy over transvaginal ultrasonography. Another major advantage of laparoscopy is the ability to directly evaluate the size of the preg-

nancy, the tubal condition, and other pathologic conditions in the pelvis. In some selected cases, even minor surgical procedures such as lysis of adhesions or tubal sterilization can be performed. The advantages of combining the local injection under direct vision with the use of the laparoscope opened a new field of treatment for early, unruptured tubal pregnancy.

METHOTREXATE. We first reported a single, local injection of methotrexate with the use of the laparoscope in 1989.[35] Methotrexate in a dose of 12.5 mg was injected locally in 27 patients with unruptured tubal pregnancies. The criteria for selection were an unruptured tube, a pregnancy diameter of <3 cm, no active bleeding, and no additional pelvic pathologic conditions. These 27 patients represented 40% of all ectopic pregnancies that were diagnosed during the study period. Twenty-four (88%) patients needed no additional surgery, whereas three patients underwent surgery because of rising titers of β-hCG with or without abdominal pain. The use of local injection enabled us to reach high concentrations of methotrexate at the pregnancy site and to avoid the systemic route; thus, possible related side effects were eliminated. Subsequent tubal patency was evaluated with hystersalpingography in 21 patients 3 months later and showed a 90% tubal patency of the affected tube.[36]

A combination of local injection with a single systemic dose of 0.5 mg/kg of methotrexate was reported during the same year[37] in an attempt to lower the systemic effect. In this report, eight of 10 patients did not need surgery, and only one patient experienced stomatitis.

PROSTAGLANDINS. The effect of prostaglandins, especially that of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on tubal function and on the corpus luteum, was recently reported.[38] It was found that $PGF_{2\alpha}$ causes increased tubal peristalsis and vasospasm of arteries in the mesosalpinx. It also showed that $PGF_{2\alpha}$ is a luteolytic agent that induces a decrease in progesterone production of the corpus luteum. This was tested by Lindblom et al.[39, 40] who injected $PGF_{2\alpha}$ (0.5 to 1.5 mg) locally into the tubal pregnancy and the corpus luteum with the laparoscope. In their small series, eight of nine patients did not require surgery. Lindblom et al. explained that the increased peristalsis of the tube caused tubal abortion of the pregnancy and that the vasospasm of the vessel prevented massive bleeding from the pregnancy site, and the luteolytic effect on the corpus luteum helped to lower hCG production.

Egarter et al.[40] used a combination of a local injection into the tube and corpus luteum of a slightly higher dosage of $PGF_{2\alpha}$ with systemic administration of PGE_2 in 18 patients and avoided surgery in 16. In their report, severe hemodynamic and cardiac side effects developed in three patients, one had massive pulmonary edema as a result of malignant hypertension and complete atrioventricular block, and the other had severe cardiac arrythmia. Those complications were explained by the massive absorption of the drug through the ovarian vasculature to the circulation. After these complications had occurred, they recommended a change in the protocol of treatment to an injection of estrogen (E_2) (instead of $PGF_{2\alpha}$) into the corpus luteum to induce a luteolytic effect.

The same research group[42] recently reported on a prospective multicenter study in which the new treatment of tubal pregnancy by means of local $PGF_{2\alpha}$ and systemic PGE_2 administration was compared with the usual surgical technique of eliminating the conceptus. Of 71 patients who were treated with prostaglandins, the method proved to be successful in 81%; 19% required surgical intervention later. The duration of hospitalization was significantly reduced in the prostaglandin group in comparison with the patients who were treated by primary surgery. In the prostaglandin group, only two of 24 subsequent hysterosalpingograms showed tubal occlusion after treatment, whereas occlusion was present in three of eight patients in the surgically treated group.

Another research group, with the original protocol of Lindblom et al.,[39] reported a 90% success rate with no side effects.[43] As in most other series with this type of therapy, the main concern is the selection of patients with emphasis on the size of the pregnancy and the initial β-hCG level. On hysterosalpingograms, tubal patency was shown to be similarly satisfactory and even better than in most other series (Table III).

An alternative to the use of prostaglandins was offered by Lang et al.[44] who performed a randomized study with laparoscopic local injection of hyperosmolar glucose in nine patients who had unruptured tubal pregnancies. They used local injection of $PGF_{2\alpha}$ combined with systemic PGE_2 in another nine patients. Better results, including fewer side effects, were noticed with the former method, and there was no need for subsequent surgery. In the prostaglandin group that required additional surgery, there was one failure and six patients reported severe abdominal pain, which was probably a result of the prostaglandin effect (Table III).

Expectant management

Ectopic pregnancy can resolve spontaneously and express only mild clinical signs or no clinical signs at all.[45] Arias Stella[46] in 1954 reported specific histologic changes in the endometrium as a result of the hCG effect, when no intrauterine pregnancy was present. In a few of these cases the source of hCG was an ectopic pregnancy, which resolved spontaneously.

Ohel et al.[47] described a patient who had minimal

Table III. Local injection in unruptured tubal pregnancy

Drug	Author	No. of cases	Success rate		Tubal patency		Side effects
			No.	%	No.	%	
Local injection (vaginal ultrasonography)							
Methotrexate	Feichtinger et al.[28, 29]	9	8	89	—	—	0
	Robertson et al.[30]	9	4	44	—	—	0
	Leeton et al.[31]	2	2	100	—	—	0
	Robertson et al.[32]	2	1	50	—	—	0
Potassium chloride	Timor Tritsch et al.[33]	1	1	100	—	—	0
PGE_2	Ribic-Paucelj et al.[34]	2	0	0	—	—	0
	Feichtinger et al.[29]	1	0	0	—	—	1
Local injection (laparoscopy)							
Local $PGF_{2\alpha}$ and systemic PGE_2	Lindblom et al.[39]	23	22	96	—	—	0
	Egarter et al.[41, 42]	71	57	81	22/24	92	3
	Vejtorp et al.[43]	11	10	91	6/7	85	0
	Lang et al.[44]	9	8	89	—	—	6
Hyperosmolar glucose	Lang et al.[44]	9	9	100	3/3	100	0
Methotrexate	Pansky et al.[35, 36]	27	24	88	19/21	90	0

clinical signs and who was diagnosed accidently as having a tubal pregnancy during laparoscopy. The pregnancy was later found in the pouch of Douglas, and there was no need for further treatment.

The fact that a considerable number of ectopic pregnancies that are diagnosed today are in a very early stage because of modern means of diagnosis, the high index of suspicion, and the fact that many of these pregnancies would have resolved spontaneously with no additional therapy form the basis of expectant management.

In 1955 Lund[48] was the first to try such an expectant management in ectopic pregnancy, and he attempted to answer two major questions: (1) What is the natural course of events and the clinical pattern of an early ectopic pregnancy when it is left untouched? and (2) When no surgery takes place and the pregnancy is reabsorbed, is there any effect on future fertility?

The diagnosis of ectopic pregnancy was based on clinical and physical examination, measurement of urinary hCG level with the Friedman technique, and curettage. Of 119 patients who entered the study, 57% did not need surgery and recovered with bed rest alone. No maternal mortality was reported, although many emergency operations were performed in the remainder of the group. In 46% of the patients who did not have surgery, an intrauterine pregnancy was reported, and in 15% ectopic pregnancy recurred.

A study of 600 Chinese women who were treated with traditional Chinese drugs or expectant management alone, was reported in the Chinese literature by Wone[49] in 1977. It is difficult to evaluate this study because few details were given concerning diagnosis and follow-up, but two cases of maternal death were reported.

Mashiach et al.[50] in 1982 reported for the first time, five patients in whom tubal pregnancy was treated with only expectant management. In three patients the diagnosis was established by laparoscopy. The criteria for selection were hemodynamic stability, β-hCG serum level <250 mIU/ml (or in a decreasing pattern), and pregnancy diameter <2 cm. One patient (20%) required a laparotomy because of abdominal pain and rising titers of β-hCG.

This series was further extended by the same research group[51] who reported on 12 of 14 patients in whom diagnoses were confirmed at laparoscopy. Only three patients required further surgery. Recently, several authors described small series of ectopic pregnancies that were treated with expectant management (Table IV). In about 60% of the 61 cases that have been reported so far, definite diagnoses were established by means of laparoscopy. The subsequent reported average success rate was 82% (range 71% to 100%). Subsequent tubal patency ranged from 70% to 100% and was no different from reports on tubal patency after other modes of therapy (Tables I and III).[22] Fertility was also covered in a few of these reports (Table IV) and showed a somewhat lower incidence of intrauterine pregnancies compared with a few recent reports of conservative surgery for tubal pregnancy.[52, 53]

There is no doubt that a certain number of ectopic pregnancies are being "overtreated" these days. Al-

Table IV. Expectant management of tubal pregnancy

Author	No. of cases	Laparoscopy for diagnosis	Success rate		Patency		Pregnancy rate	
			No.	%	No.	%	No.	%
Ohel et al.[47]	1	Yes	1	100	1/1	100	—	—
Carp et al.[51]	14	12 only	11	79	5/5	100	4/5	80
Derricks et al.[55]	12	No	11	89	—	—	—	—
Garcia et al.[56]	13	No	12	92	7/10	70	5/12	42
Fernandez et al.[57]	14	Yes	10	71	6/6	100	3/6	50
Sauer et al.[14]	5	Yes	5	100	4/5	80	—	—
TOTAL	61		50	82	23/27	85	12/23	52

though we have sophisticated means of early diagnosis, it is still very difficult to determine safe and effective criteria at diagnosis, which will help to differentiate between those pregnancies that are destined to be absorbed spontaneously and those that will continue to grow and finally rupture the tube. Reliance on the clinical presentation alone (as was done by Lund[48]) will result in large numbers of emergency operations and irreversible damage to about 50% of patients. Use of β-hCG levels will postpone treatment by at least 24 to 48 hours,[14] and more than that, a decrease in amounts of β-hCG is not always a guarantee of complete resolution.[54]

We think that expectant management is appropriate in a very small number of patients, who meet the strict criteria of minimal clinical complaints, a definite pattern of decreasing β-hCG levels, and laparoscopic confirmation of a small, unruptured, and nonbleeding tubal pregnancy.

Comment

The clinical picture of ectopic pregnancy has dramatically changed in recent years from an emergency situation and a life-threatening condition, in most cases, to a much more benign condition with milder presentation. Because patients are usually in excellent clinical condition and hemodynamically stable, many investigators were able to successfully treat many of them with new and less invasive procedures and to achieve more than satisfactory results. In an analysis of these methods, we found that the success rates of most of them are practically equal (80% to 90%) when compared with those few reports of expectant management alone. It seems that local injection under ultrasonographic control is an exception that shows somewhat less promising results. This is probably because of suboptimal visualization of the gestational sac and thus reduced accuracy in localization of the injection site.

The selection criteria that were used by most investigators including small-sized pregnancies, low β-hCG levels, and the absence of fetal pulse, probably mean that many of these ectopic gestations were nonviable and would have resolved spontaneously regardless of the method of treatment used.

The main question that remains to be answered is how to determine, during the evaluation of a case of ectopic pregnancy, whether it is growing or destined to resolve spontaneously. We believe that at least two consecutive β-hCG determinations are needed to establish a rising trend before any treatment is started. Expectant management is probably the treatment of choice when no such trend is evident. However, when such a rise in β-hCG level is found, a prospective randomized study that compares the various nonsurgical measures may show which treatment modality is the safest and most efficient.

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Serum levels of inhibin in maternal and umbilical blood during pregnancy

Toru Tabei, MD,[a] Kazuhiko Ochiai, MD,[b] Yoshiteru Terashima, MD,[b] and Naoki Takanashi, PhD[c]

Kawagoe and Tokyo, Japan

Inhibin levels were measured by a double antibody heterologous radioimmunoassay in the peripheral serum of 75 pregnant women throughout gestation and in serum from the umbilical vein and artery, which was obtained at the time of delivery. For reference, samples were obtained from 20 nonpregnant women in the early (days 0 to 3), mid (days 4 to 8), and late (days 9 to 14) luteal or follicular phase. Maternal serum levels of inhibin (mean ± SEM) in early (6 to 12 weeks) gestation (36.4 ± 2.6 U/ml, $n = 36$) were significantly ($p < 0.01$) higher than those in serum from nonpregnant women in the mid (23.9 ± 2.5 U/ml, $n = 19$) or late (11.3 ± 0.6 U/ml, $n = 19$) luteal phase. Inhibin levels in maternal serum fell to 15.9 ± 1.4 U/ml ($n = 24$) in mid (14 to 20 weeks) gestation and then gradually increased during late (21 to 40 weeks) gestation to peak levels of 49.4 ± 5.1 U/ml ($n = 9$) at 36 to 37 weeks. Inhibin levels declined in parallel with human chorionic gonadotropin concentrations during the first trimester ($r = 0.587$ at $p < 0.01$). Significant positive correlations ($p < 0.001$) were observed between serum levels of inhibin and 17β-estradiol ($r = 0.560$), progesterone ($r = 0.648$), and human placental lactogen ($r = 0.715$) during mid and late (20 to 40 weeks) gestation. Inhibin levels in umbilical vein serum (38.5 ± 1.3 U/ml, $n = 5$) were not different from those in umbilical artery serum (39.4 ± 3.6 U/ml) but were significantly ($p < 0.01$) lower than those in maternal serum (50.9 ± 5.3 U/ml), which was obtained at the time of delivery. By day 5 of puerperium, serum levels of inhibin in the maternal vein were extremely low (2.3 ± 0.1 U/ml, $n = 7$); these levels were nearly one fifth lower than follicular phase levels of 10.9 ± 3.4 U/ml ($n = 38$). We propose that maternal inhibin in early gestation is secreted from the corpus luteum of pregnancy but that increasing inhibin levels during mid and late gestation result from inhibin that is produced by the placenta. The lack of an umbilical arterial-venous gradient for inhibin and the higher levels of inhibin in maternal serum argue against a fetal source of inhibin in the maternal circulation. The physiologic function of inhibin that is produced by the corpus luteum and by the placenta remains to be determined. (Am J Obstet Gynecol 1991;164:896-900.)

Key words: Inhibin, pregnancy, placenta, umbilical blood

Inhibin, a glycoprotein hormone that is secreted by the ovaries and testes, is a regulator of pituitary follicle-stimulating hormone secretion.[1, 2] The molecule has been purified and characterized as a heterodimeric protein with α- and β-subunits.[3] A specific and sensitive heterologous radioimmunoassay for the measurement of the circulating serum levels of human inhibin has been developed,[4] and results during normal menstrual cycle,[5] in early normal pregnancy,[6] and in three pregnancies in women with ovarian failure throughout gestation[7] have been reported. In normal women inhibin levels rise during the luteal phase in parallel with progesterone levels,[5] which suggests that inhibin is produced by the corpus luteum.

Recently, inhibin bioactivity and immunoactivity have been demonstrated in the human placenta, which suggests that inhibin is produced by the human placenta during pregnancy.[7-10] Inhibin gene expression[11] and messenger ribonucleic acid (mRNA) for the α-subunit of inhibin[12] have also been demonstrated in the human placenta. In addition, elevated circulating immunoactive inhibin levels were found in women with complete hydatidiform mole during early pregnancy.[13]

There are presently no reports that describe the

From the Department of Obstetrics and Gynecology, Sekishindo Hospital, Kawagoe,[a] The Jikei University School of Medicine, Tokyo,[b] and Special Reference Laboratory, Inc., Tokyo.[c]
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Reprint requests: Toru Tabei, MD, Department of Obstetrics and Gynecology, Sekishindo Hospital 25-19 Wakitahoncho, Kawagoe City, Saitama, 350 Japan.
6/1/26277

physiologic characteristics of inhibin secretion throughout pregnancy. Therefore we measured the concentrations of circulating inhibin in maternal serum throughout gestation and in umbilical vein and artery serum that was obtained at the time of delivery.

We have also assessed the relationship between levels of inhibin in maternal serum and those of 17β-estradiol, progesterone, human placental lactogen, and human chorionic gonadotropin, all of which are produced by the human placenta during gestation.

Patients and methods

Serum samples were obtained on 158 occasions from the peripheral vein of 75 pregnant women throughout gestation (6 to 40 weeks), from the umbilical vein and artery of five pregnant women at the time of delivery, and on 87 occasions from the peripheral vein of 20 nonpregnant women with normal ovulatory cycles.

The menstrual cycle of nonpregnant women was divided into six phases: early (day 14 to day 9), mid (day 8 to day 4), and late (day 3 to day 0) follicular phases and early (day 0 to day 3), mid (day 4 to day 8), and late (day 9 to day 14) luteal phases. The day of ovulation was designated as day 0 from charts of daily basal body temperature. Gestational age of pregnant women was calculated from the last menstrual period and/or reevaluated by ultrasonographic findings. Serum was frozen and stored for measurement of inhibin, 17β-estradiol, progesterone, human placental lactogen and human chorionic gonadotropin by radioimmunoassay or enzyme immunoassay.

Serum levels of inhibin were measured by a double antibody heterologous radioimmunoassay with iodine 125-labeled purified 31 kd inhibin from bovine follicular fluid and rabbit antiserum to 31 kd bovine inhibin, which was supplied by Drs. D.M. DeKretser and D.M. Robertson of Monash University in Australia. Serum samples (0.1 ml) were assayed in duplicate. The assay standard was World Health Organization porcine inhibin. This standard was assigned a unitage of 2000 U per ampule by the World Health Organization working group. Parallelism was observed between dose-response lines of the World Health Organization inhibin standard, human serum, and human follicular fluid. The specific binding of ^{125}I-inhibin (bound/total; B/T) was 35% in the radioimmunoassay. The minimum detectable dose was 2 U/ml with a 95% confidence interval. The median effective dose was 18 U/ml. The intraassay and interassay coefficients of variation were 3.8% and 7.8%, at 17.3 U/ml, respectively.

Serum concentrations of human chorionic gonadotropin were determined by enzyme immunoassay with reagents that were supplied by Takeda Pharmaceutical Co., Osaka.[14] Serum concentrations of 17β-estradiol,[15] progesterone,[16] and human placental lactogen[17] were measured by radioimmunoassay with reagents that were supplied by Japan D.P.C. Corp., Tokyo, and Pharmacia, Uppsala.

Statistical analyses were performed by comparison of the date with Student's t test, one-way analysis of variance, and linear regression analysis. Groups were considered to be significantly different at a level of $p < 0.01$ or $p < 0.001$. Data were expressed as the mean ± SEM.

Results

Serum levels of inhibin (mean ± standard error of the mean, U/ml) throughout the normal menstrual cycle were similar during the early (9.0 ± 0.4 U/ml, $n = 11$), mid (11.6 ± 1.0 U/ml, $n = 16$), and late (11.1 ± 0.6 U/ml, $n = 11$) follicular phases. Serum levels of inhibin were increased during the early luteal phase (16.5 ± 3.8 U/ml, $n = 8$), reached maximum levels of 23.9 ± 2.5 U/ml ($n = 19$) in the mid luteal phase, and declined in the late luteal phase to 11.3 ± 0.6 U/ml ($n = 19$). The serum levels of inhibin in early (6 to 12 weeks) gestation (36.4 ± 2.6 U/ml, $n = 36$) were significantly ($p < 0.01$) higher than those in the mid and late luteal phases of nonpregnant women.

Circulating maternal levels of inhibin at weekly intervals throughout pregnancy are compared with those of 17β-estradiol, progesterone, human placental lactogen, and human chorionic gonadotropin throughout gestation in Fig. 1. Fig. 1, *a*, shows that serum levels of inhibin were elevated during early (6 to 19 weeks) gestation with peak levels of 45.1 ± 5.2 U/ml ($n = 12$) at 8 to 9 weeks, declined to lower levels (15.9 ± 1.4 U/ml, $n = 24$) in mid (14 to 20 weeks) gestation, and then progressively increased with gestational age during mid and late (20 to 40 weeks) gestation to peak levels of 49.4 ± 5.1 U/ml ($n = 9$) at 36 to 37 weeks. The pattern of increase in serum inhibin during mid to late gestation was similar to that of 17β-estradiol (Fig. 1, *b*), progesterone (Fig. 1, *b*) and human placental lactogen (Fig. 1, *c*) but differed from that of human chorionic gonadotropin (Fig. 1, *c*). Significant positive correlations were observed between circulating inhibin and 17β-estradiol ($r = 0.560$ at $p < 0.001$, $n = 103$) (Fig. 2), progesterone ($r = 0.648$ at $p < 0.001$, $n = 104$) (Fig. 3), and human placental lactogen ($r = 0.715$ at $p < 0.01$, $n = 103$) (Fig. 4) during mid and late gestation. Although no significant correlation ($r = 0.329$, $n = 103$) was found between inhibin and human chorionic gonadotropin concentrations between 20 and 40 weeks, a striking correlation was observed in the period of 6 to 20 weeks' gestation ($r = 0.587$ at $p < 0.001$, $n = 56$).

The levels of inhibin (mean ± SEM, U/ml) in maternal vein serum samples that were obtained from five pregnant women at the time of delivery (50.9 ± 5.3

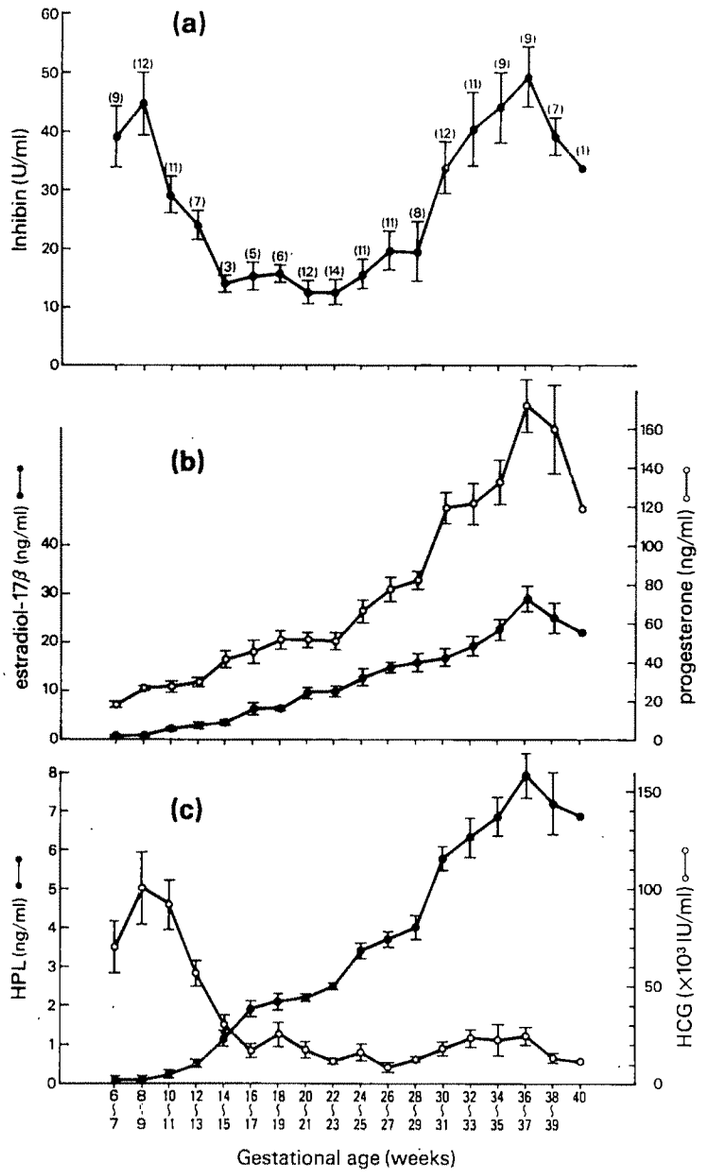


Fig. 1. Serum levels (mean ± SEM) of inhibin (units per milliliter), 17β-estradiol (nanograms per milliliter), progesterone (nanograms per milliliter), human placental lactogen *(HPL)* (nanograms per milliliter), and human chorionic gonadotropin *(HCG)* ($\times 10^3$ IU/ml), which were obtained from 75 pregnant women throughout gestation (6 to 40 weeks). Total number of serum samples was 158. *Values in parentheses* refer to numbers of serum samples for measurement.

U/ml) were significantly ($p < 0.01$) higher than those in samples that were obtained from the umbilical vein (38.5 ± 1.3 U/ml) and the umbilical artery (39.4 ± 3.6 U/ml). There was no significant ($p < 0.01$) difference between the umbilical venous and umbilical arterial concentrations. In addition, the serum levels of inhibin in maternal vein serum, which was obtained from seven mothers at day 5 of puerperium, were extremely low (2.3 ± 0.1 U/ml). The levels were approximately one fourth less than those in normal women during the follicular phase, 10.9 ± 3.4 U/ml ($n = 38$).

Comment

This study demonstrates that circulating levels of immunoactive inhibin are elevated during early gestation, gradually decline to lower levels in mid gestation, and then increase with gestational age to peak levels at 36 to 37 weeks. This pattern of increase in serum inhibin

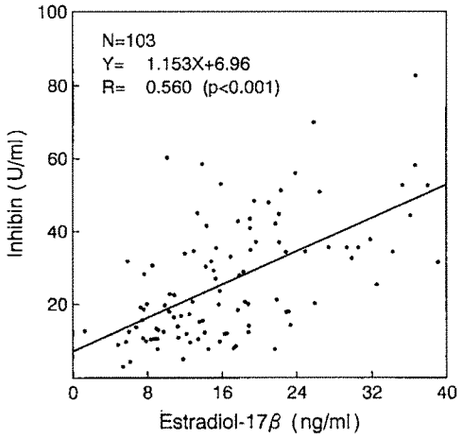


Fig. 2. Relation between serum levels of inhibin and those of 17β-estradiol during mid and late gestation (20 to 40 weeks); $r = 0.560$ at $p < 0.001$.

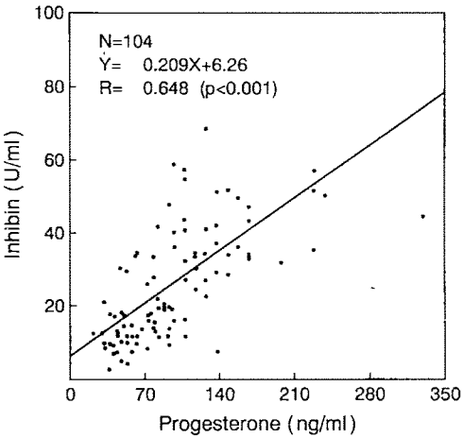


Fig. 3. Relation between serum levels of inhibin and those of progesterone during mid and late gestation (20 to 40 weeks); $r = 0.648$ at $p < 0.001$.

during mid and late gestation is similar to the patterns of increase of 17β-estradiol, progesterone, and human placental lactogen, all of which are produced by the human placenta. By contrast, inhibin levels paralleled those of human chorionic gonadotropin in early gestation.

Previous studies have demonstrated biologic and immunologic activity of inhibin[8-10] and inhibin α-subunit mRNA[12] in the human placenta. In addition, elevated levels of inhibin throughout gestation were reported in three pregnant women without ovarian tissue.[7] These data and our present findings suggest that the placenta is an important source of inhibin during mid and late pregnancy. The positive correlation between inhibin levels and the concentration of 17β-estradiol, progesterone, and human placental lactogen in mid to late pregnancy suggests that a common mechanism governs the synthesis and secretion of these placental steroids and proteins. The concentration of mRNA per cell for human placental lactogen is believed to remain relatively constant during pregnancy,[18] which suggests that the increase in total placental human placental lactogen mRNA[19] is due to an increase in the number of syncytial cells that express the human placental lactogen gene rather than to a hormonal factor that regulates human placental lactogen mRNA. A similar mechanism may explain the concomitant increase of inhibin secretion. However, a paracrine effect of inhibin cannot be excluded.

A fetal source of inhibin is unlikely because of the higher levels in maternal serum than those in umbilical blood serum that was obtained at the time of delivery and because of the absence of an umbilical venous/arterial difference.

On the other hand, the source of elevated inhibin

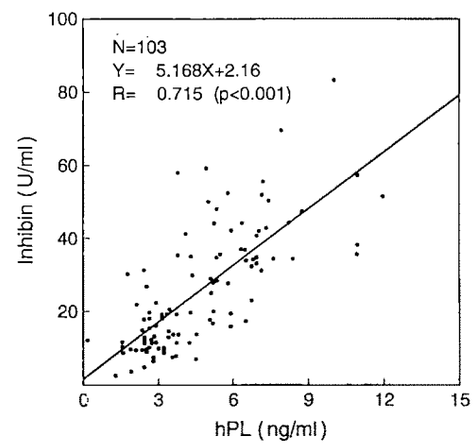


Fig. 4. Relation between serum levels of inhibin and those of human placental lactogen *(hPL)* during mid and late gestation (20 to 40 weeks); $r = 0.715$ at $p < 0.001$.

during early pregnancy may be from the maternal corpus luteum or the placenta. The gradual decrease in inhibin levels, which paralleled that of human chorionic gonadotropin concentrations during early pregnancy in normal pregnant women, together with the absence of this pattern in pregnant women with ovarian failure and oocyte transfer,[7] suggests that the ovary is the source of high inhibin levels in maternal serum during the first trimester. Inhibin α-subunit mRNA has been found in the corpora lutea of primates,[20] and ablation of the corpus luteum leads to an abrupt decline in circulating inhibin levels.[21] The finding that a gonadotropin releasing hormone antagonist abruptly reduced inhibin secretion in the luteal phase of nonhuman

primates[22] indicates that pituitary gonadotropins regulate the secretion of inhibin by the corpus luteum. Thus it is likely that human chorionic gonadotropin also stimulates inhibin production through an action on the ovary during pregnancy. The ovary, however, is not the only major source of inhibin production in early pregnancy since inhibin was present in the serum of three pregnant women who had no ovarian function.[7] Thus further studies are required to define the source and regulation of the elevated circulating inhibin during pregnancy.

A significant difference between bioactive and immunoactive inhibin in the human placenta and ovaries[9] may reflect the secretion of isoforms of inhibin with variable biologic activities.[23] A bioactive/immunoactive difference was also observed in human serum.[24] We have not yet examined the bioactivities of inhibin that is produced during pregnancy. The physiologic role of placental inhibin is presently unknown. Potential functions of placental inhibin may include the endocrine control of gonadotropin secretion by the mother or fetus or the paracrine actions within the fetoplacental unit.

Since inhibin is secreted into the maternal circulation by the human placenta in parallel with other placental hormones during mid and late gestation, measurement of this substance may serve as an index of placental function.

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Restriction fragment length polymorphism analysis to study the genetic origin of complete hydatidiform mole

Tsang-Ming Ko, MD, Chang-Yao Hsieh, MD, Hong-Nerng Ho, MD, Fon-Jou Hsieh, MD, and Tzu-Yao Lee, MD

Taipei, Taiwan, Republic of China

To determine the genetic origin of the complete hydatidiform mole, 20 abnormal pregnancies were studied with restriction fragment length polymorphism with five genomic probes: EJ 6.6, β-globin gene, 3′α-hypervariable region, J-Bir, and St14. In the 12 cases of molar pregnancy, pure paternal origin was proved in 11 cases, but both maternal and paternal inheritance were shown in only one case. In the cases with pure paternal origin, all of the restriction fragment length polymorphisms were homozygous, although those of the fathers were heterozygous at 15 loci. In the four cases that mimicked hydatidiform mole but were diagnosed as hydropic change of villi, both paternal and maternal inheritance were noted. In the four pregnancies with blighted ovum, both paternal and maternal inheritance were shown in three cases; and in one case with a balanced translocation between chromosomes 13 and 14, only paternal inheritance was noted. This study showed that most of the complete hydatidiform moles were caused by fertilization of an empty egg by a duplicated haploid sperm, but rare exceptions may exist. (Am J Obstet Gynecol 1991;164:901-6.)

Key words: Complete hydatidiform mole, restriction fragment length polymorphism, genetic origin

Complete hydatidiform mole is an abnormal pregnancy that is characterized by swollen chorionic villi and trophoblastic hyperplasia, and it has a propensity to malignancy.[1] Most of the cases of complete hydatidiform mole have 46,XX chromosome complement, and only a few have 46,XY. The genetic origin of the complete hydatidiform mole has been studied by means of chromosome heteromorphism and/or analysis of polymorphic enzymes of the molar tissue and the parental blood.[2-9] Most of the 46,XX moles are derived from fertilization of an "empty" egg by a duplicated haploid sperm,[2, 4, 8, 9] whereas the 46,XY moles are caused by dispermy.[3, 5, 6] However, chromosome heteromorphism study might be subject to technical variation, and the reliability of subjective assessment of the visible chromosome heteromorphism has been questioned.[10] Evaluation by other means would be useful for further confirmation of the previous observation.

The recent advance in molecular biology has enabled us to use deoxyribonucleic acid (DNA) markers to trace the parental origin of many conditions, such as Down syndrome, Edwards syndrome, and the issue of paternity.[11, 12] In genomic DNA, one mutation occurs in about every 300 to 500 nucleotides. Some of the mutations affect the recognition site of certain restriction enzymes, which generates two-allelic restriction fragment length polymorphism when the DNA is digested with a particular enzyme. In addition, there are many regions in the genome, which are composed of repeating sequences that are arranged in tandem and that result in highly polymorphic multiallelic restriction fragment length polymorphisms because of the insertion-deletion of these sequences. These site and length polymorphisms are useful and reliable markers in linkage analysis for the prenatal diagnosis of single-gene disorders[13-15] and for the identification of genetic origin of many conditions.[11, 12]

Taiwan has a relatively high incidence of complete hydatidiform mole.[16] Recently, it was estimated that about 1 in 500 pregnancies turns out to be complete hydatidiform mole. The mechanisms of complete hydatidiform mole development are still not completely clear. Although, in most cases, the genetic origin of complete hydatidiform mole has been shown to be paternal by chromosome heteromorphism study, further confirmation by restriction fragment length polymorphism analysis will be very valuable. For this purpose, we have collected 12 histologically confirmed cases of complete hydatidiform mole and 8 cases of other pregnancy wastage; we have studied five restriction fragment length polymorphisms of these conceptions with genomic probes from three chromosomes.

From the Department of Obstetrics and Gynecology, College of Medicine, National Taiwan University.
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Reprint requests: Tsang-Ming Ko, MD, 290, No. 1, Chang-Te St., National Taiwan University Hospital, Taipei, Taiwan, 10016, Republic of China.
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Table I. Restriction fragment length polymorphisms generated by the DNA probes and the restriction enzymes used

Probe name	*Map position*	*Restriction enzyme*	*Fragment length (kb)*
EJ 6.6	11p15.5	*Pvu* II	2.6; 3.6; 4.2
β-Globin	11p15.5	*Ava* II	2.0; 2.2
3′α-HVR	16p13.3	*Bgl* II	5 to 10 (about 7 alleles)
J-Bir	Xp21.1-21.3	*Bam* HI	5.6; 21
St14	Xq28	*Taq* I	3.4 to 6.6 (8 alleles)

Patients and methods

Sixteen conceptuses with grossly vesicular change of the chorionic villi and four conceptuses with normal-appearing chorionic villi were collected in this study. All of the conceptus products were obtained aseptically and were usually divided into three parts. The first part was fixed in formalin-alcohol for histologic examination; the second part was set up in tissue culture for cytogenetic analysis; and the third part was directly extracted for DNA for restriction fragment length polymorphism study.

Family 1 requires some description. A 29-year-old woman, gravida 5, para 2, came to our hospital at 15 weeks' gestation; she complained of intermittent vaginal spotting that had lasted for about 2 months. Physical examination showed hypertension (186/110 mm Hg), lower-leg edema, and a large-for-date uterus at umbilical level. Ultrasonographic examination showed a normal-appearing fetus with active fetal heart motion. The biparietal diameter of the fetus was 3.1 cm, which is compatible with the gestational age. A normal placenta was located at the anterior uterine wall. However, more than half of the intrauterine volume was occupied by a complex echogenic and echolucent mass that was compatible with a molar pregnancy. The placenta and the vesicular tissues were clearly separable. Because of severe hypertension and the ultrasonographic findings, termination of the pregnancy was advised. While the patient was under epidural anesthesia and continuous ultrasonic guidance, about 660 gm of vesicular tissue was suctioned out. The fetus and the placenta were evacuated in fragments. The fetus measured about 10 cm from crown to feet and weighed about 20 gm. The placenta measured about 10 × 6 × 0.5 cm and weighed about 22 gm. No vesicular change was noted in the placenta. The molar tissue and placenta fragments were obtained for both cytogenetic and DNA analysis.

For tissue culture, the decidual tissues were removed under a dissecting microscope. The gestational products were then treated with trypsin-collagenase to yield small tissue fragments, which were cultured with Chang's medium in an incubator at a temperature of 37° C with 5% carbon dioxide. Routine G-banding was used in the determination of karyotypes. In a few cases in which cytogenetic study failed or was not done, determination of the sex of the conceptus was performed by dot hybridization with Y-specific probe pY3.4.[17]

For restriction fragment length polymorphism analysis, in addition to the gestational tissue, white blood cells from both parents were also needed. From each parent, 10 ml of venous blood was obtained and anticoagulated with ethylenediaminetetraacetic acid. The buffy coat was separated for DNA extraction, and the maternal plasma was used for quantitation of the β-subunit of human chorionic gonadotropin.

Methods for DNA extraction were described previously.[14] Briefly, the molar and villous tissues were pulverized after they had been frozen in liquid nitrogen. The tissue powder and buffy coat were treated with lysing buffer, followed by ribonuclease A and then proteinase K digestion. DNA was purified by phenol-chloroform extraction, extensive dialysis, and ethanol precipitation.

Five genomic probes, two (EJ 6.6 and β-globin gene) from chromosome 11,[14] one (3′ hypervariable region of α-globin gene [3′α-HVR]) from chromosome 16,[18] and two (J-Bir and St14) from the X chromosome (the former from the short arm and the latter from the long arm),[19, 20] were used for restriction fragment length polymorphism study (Table I). β-Globin gene and J-Bir probes detected two-allelic polymorphisms, and the other probes detected multiallelic polymorphisms.

For each restriction fragment length polymorphism study, 5 μg genomic DNA was digested with 15 to 20 U restriction enzyme (Boehringer Mannheim, Penzberg, Federal Republic of Germany; Bethesda Research Laboratories, Gaithersburg, Md., and Toyobo Co., Ltd., Osaka, Japan) under the conditions recommended by the manufacturers. The DNA fragments were size fractionated by 0.8% agarose gel electrophoresis and then transferred to nylon filter (Zetabind, Cuno Inc., Meriden, Conn.) by Southern blotting.[21] After baking in a vacuum, the filter was prehybridized and then hybridized with a phosphorus 32-labeled radioactive probe, which was prepared by nick-translation or random-primer method.[22, 23] The filter was washed under stringent conditions and then autoradiographed for 3 to 7 days at a temperature of −70° C as described previously.[14] For the two-allelic restriction fragment length polymorphisms, (+) was designated for the presence of the polymorphic site and (−) was designated for the absence of the site. For the multiallelic restriction fragment length polymorphisms Arabic numerals were used in each family for distinction of the different alleles; the same numeral from different families did not necessarily represent the same allele. The maternal β-human chorionic gonadotropin levels were

Table II. The cytogenetic study, β-human chorionic gonadotropin level, restriction fragment length polymorphism, and histologic evaluation of the whole series

Family No.	*Family member*	*Cytogenetics*	*β-hCG (mIU/ml)*	*EJ 6.6*	*β-globin gene*	*3'α-HVR*	*J-Bir*	*St14*	*Histologic evaluation*
1	M		58,100	2/2	+/+	1/3	+/+	1/2	
	F			1/2	+/−	2/4	+	1	
	CHM	46,XX		1/1*	−/−*	2/2*	+/+	1/1	CHM
	Fetus	46,XY		1/2	+/−	1/2	+	2	
2	M		>100,000	1/1	+/−	3/3	ND	1/2	
	F			1/1	ND	1/2	—	3	
	CHM	46,XX		1/1	−/−	2/2*	−/−	3/3	CHM
3	M		>100,000	1/1	+/−	2/3	+/−	1/1	
	F			1/1	−/−	1/3	+	2	
	CHM	46,XX		1/1	−/−	3/3*	+/+	2/2	CHM
4	M		>100,000	1/3	+/−	1/1	+/−	1/2	
	F			2/3	+/+	2/3	−	3	
	CHM	46,XX		2/2*	+/+	3/3*	−/−	3/3	CHM
5	M		>100,000	ND	+/−	1/3	−/−	1/2	
	F			ND	−/−	2/4	+	3	
	CHM	Y(−)		ND	−/−	2/2*	+/+	3/3	CHM
6	M		240,000	1/3	ND	1/3	ND	1/1	
	F			2/3	ND	2/4	ND	2	
	IM	46,XX		3/3*	ND	2/2*	ND	2/2	IM
7	M		1,725	1/1	+/−	1/2	+/−	1/3	
	F			1/1	−/−	2/2	−	2	
	CHM	46,XX		1/1	−/−	2/2	−/−	2/2	CHM
8	M		>100,000	1/2	+/−	1/2	+/−	1/1	
	F			1/1	+/−	1/1	−	2	
	CHM	46,XX		1/1	+/+*	1/1	−/−	2/2	CHM
9	M		>100,000	1/2	−/−	1/1	+/−	1/1	
	F			3/3	−/−	2/3	+	2	
	CHM	46,XX		3/3	−/−	3/3*	+/+	2/2	CHM
10	M		210,000	1/1	+/+	1/3	+/−	1/2	
	F			1/1	+/−	1/2	−	3	
	CHM	46,XX		1/1	−/−*	2/2*	−/−	3/3	CHM
11	M		54,418	1/1	+/+	1/2	ND	ND	
	F			1/1	−/−	1/3	ND	ND	
	CHM	46,XX		1/1	−/−	1/1*	ND	ND	CHM
12	M		>100,000	1/1	−/−	1/3	+/−	1/2	
	F			1/2	−/−	2/4	+	3	
	CHM	46,XX		1/2	−/−	3/4	+/+	1/3	CHM
13	M		>100,000	1/2	+/−	1/2	ND	1/1	
	F			1/2	+/−	3/3	ND	2	
	A	47,XXY		1/2	+/−	1/3	ND	1/2	HV
14	M		18,835	ND	+/−	1/1	ND	1/1	
	F			ND	+/−	2/2	ND	2	
	A	Y(−)		ND	+/−	1/2	ND	1/2	HV
15	M		66,480	1/1	+/−	1/2	ND	1/1	
	F			1/1	+/−	3/4	ND	2	
	A	46,XX		1/1	+/+	1/3	ND	1/2	HV
16	M		73,860	ND	−/−	1/2	ND	1/2	
	F			ND	+/−	1/3	ND	2	
	A	ND		ND	−/−	2/3	ND	1/2	HV
17	M		53,639	ND	+/+	1/2	ND	1/1	
	F			ND	−/−	1/3	ND	1	
	A	45,XX,−13,−14 +t(13q,14q)		ND	−/−	3/3*	ND	1/1	GT
18	M		2,300	1/2	ND	1/2	+/−	1/1	
	F			1/3	ND	3/4	+	2	
	A	45,X		1/2	ND	1/3	+	2	GT
19	M		6,900	ND	+/−	1/2	−/−	1/1	
	F			ND	+/−	3/4	−	2	
	A	ND		ND	+/+	1/3	−/−	1/2	GT
20	M		7,680	ND	−/−	2/3	ND	1/2	
	F			ND	+/+	1/1	ND	3	
	A	46,XX		ND	+/−	1/3	ND	1/3	GT

β-hCG, β-Human chorionic gonadotropin; *M*, mother; *F*, father; *ND*, not done; *CHM*, complete hydatidiform mole; *IM*, invasive mole; *A*, abortus; *HV*, hydropic villi; *GT*, gestational tissue.

*Homozygous restriction fragment length polymorphism in conceptus and heterozygous restriction fragment length polymorphism in its father; *underline*, restriction fragment length polymorphism that shows pure paternal origin.

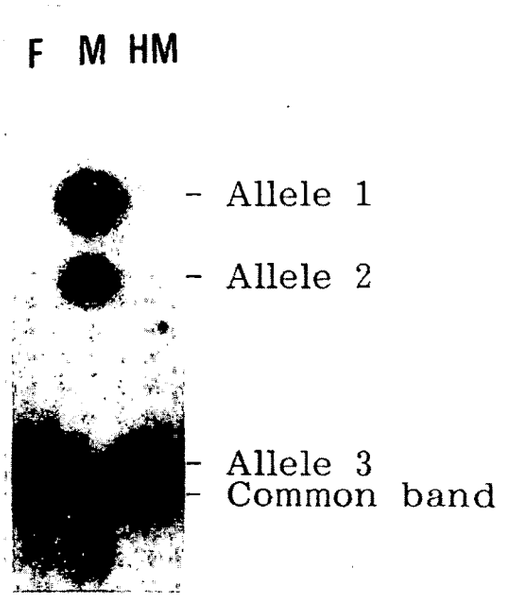


Fig. 1. Autoradiograph of DNA digestion with *Pvu* II and hybridization with probe EJ 6.6 in family 9. *M*, Mother; *F*, father; *HM*, hydatidiform mole.

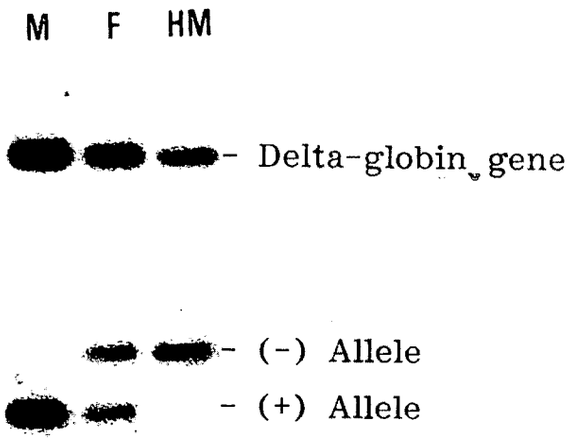


Fig. 2. Autoradiograph of DNA digestion with *Ava* II and hydridization with β-globin gene probe in family 10. The δ-globin gene cross-hybridized with the probe. Abbreviations as in Fig. 1 legend.

quantitated by enzyme-linked immunoassay (Abbott Laboratories, Chicago).

Results

In the 16 cases with grossly swollen chorionic villi, 11 were diagnosed as complete hydatidiform mole, one as invasive mole (family 6), and the other four as hydropic change of villi by histologic conditions. Family 1 had twin pregnancy with one twin that developed to complete hydatidiform mole.

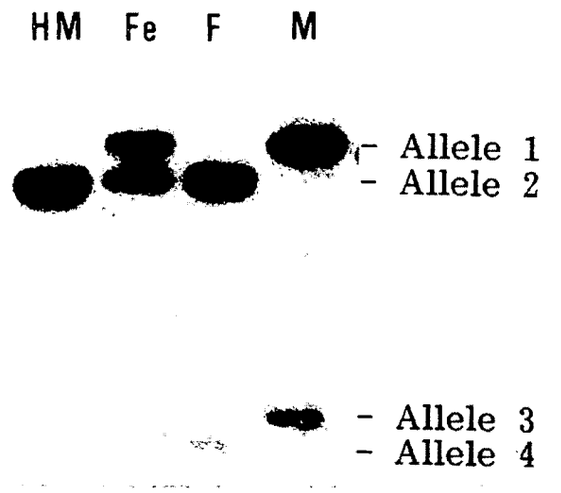


Fig. 3. Autoradiograph of DNA digestion with *Bgl* II and hybridization with probe 3′α-HVR in family 1. *Fe*, Fetus; other abbreviations as in Fig. 1 legend.

The results of cytogenetic study, β-human chorionic gonadotropin quantitation, restriction fragment length polymorphism analysis, and histologic study of the whole series are summarized in Table II. In the 12 confirmed cases of molar pregnancy, 11 were 46,XX; however, cytogenetic study was not done in family 5, but DNA dot hybridization showed absence of the Y chromosome.

Of the 12 cases of molar pregnancy, 11 (families 1 to 11) originated from the paternal side only as shown by restriction fragment length polymorphism (Table II, Figs. 1 to 5). All of the loci in molar tissue were homozygous, although 15 loci in the fathers were heterozygous. In family 12, the mole had both paternal and maternal complements as revealed by 3′α-HVR and St14 probes. In addition, restriction fragment length polymorphism at EJ 6.6 was also heterozygous, which is consistent with the paternal and maternal inheritance. The maternal β-human chorionic gonadotropin level was also highly elevated. After suction curettage, the patient had an uneventful course and conceived again about 1 year later.

In contrast to the first 11 cases of molar pregnancy, the four conceptions (families 13 to 16) that mimicked complete hydatidiform mole in gross appearance but were diagnosed as hydropic change of chorionic villi by histologic evaluation showed both paternal and maternal inheritance. In the other four pregnancies, which were clinically diagnosed as blighted ovum, both paternal and maternal inheritance were shown in three

cases (families 18 to 20). However, in case 17, cytogenetic study showed a balanced translocation between chromosomes 13 and 14, (45,XX,-13,-14,+t[13q,14q]); whereas the karyotypes of the peripheral blood in both parents were normal. Restriction fragment length polymorphism analysis showed pure paternal inheritance at the β-globin gene and 3′α-HVR loci. In addition, at the latter locus, the father was heterozygous and the conceptus was homozygous.

Comment

Restriction fragment length polymorphism analysis is very useful for tracing the genetic origin of many conditions.[11-15] In this study, 11 (92%) of the 12 cases of molar pregnancy inherited the genetic materials only from the paternal side; no maternal genome contributed to the conception products. This finding suggested that complete hydatidiform mole might originate from an empty egg that is fertilized by several mechanisms: (1) by two haploid sperm; (2) by a diploid sperm because of first meiosis failure; (3) by a diploid sperm because of second meiosis failure; and (4) by a haploid sperm, which then duplicates to produce a diploid chromosome complement. In this study, all of the restriction fragment length polymorphisms in the molar tissue were homozygous, even though 15 loci in the fathers were heterozygous (Table II). This fact does not favor the mechanism of fertilization by two haploid sperm or by a diploid sperm as a result of first meiosis failure. On the other hand, the three autosomal genomic probes, EJ 6.6, β-globin gene, and 3′α-HVR, are located at the terminal portions of chromosomes 11 and 16, respectively (Table I)[24]; hence DNA recombinations are very likely to occur during meiosis, and heterozygosity at some of these restriction fragment length polymorphisms is expected to be found in a diploid sperm as a result of second meiosis failure. However, no heterozygous restriction fragment length polymorphisms were detected in the first 11 cases of molar pregnancy. Hence the mechanism of fertilization of an empty egg by a haploid sperm, which then duplicates without cytokinesis to restore diploid number, seems most compatible with our observations and other previous reports.[2,4,8,9] Recently, Litt et al.[25] with probes for three highly polymorphic, variable-number tandem-repeat loci, also demonstrated homozygosity of complete hydatidiform mole. Saji et al.[26] with a minisatellite DNA probe, confirmed the androgenesis of complete hydatidiform mole. In mouse experiments the paternal genetic materials were found to be responsible for the growth of trophoblast, whereas the maternal genetic materials are needed for proper embryo development.[27,28] This animal model could explain the association of complete hydatidiform mole with pure androgenetic inheritance. However, in case 12, both maternal and paternal inheritance were noted as revealed by 3′α-HVR and St14 probes (Table II). The maternal β-human chorionic gonadotropin level and the cytogenetic study, gross appearance, and histologic evaluation of the conceptus were typical for a diagnosis of complete hydatidiform mole. The mechanism of maternal contribution to the genesis of complete hydatidiform mole needs further investigation.

In other types of pregnancy wastages, such as hy-

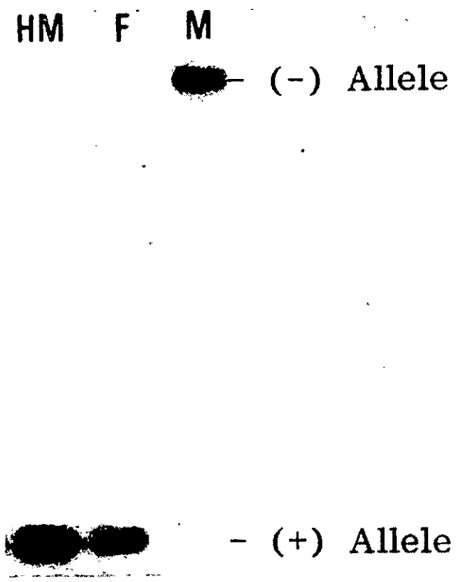


Fig. 4. Autoradiograph of DNA digestion with *Bam*HI and hybridization with probe J-Bir in family 5. Abbreviations as in Fig. 1 legend.

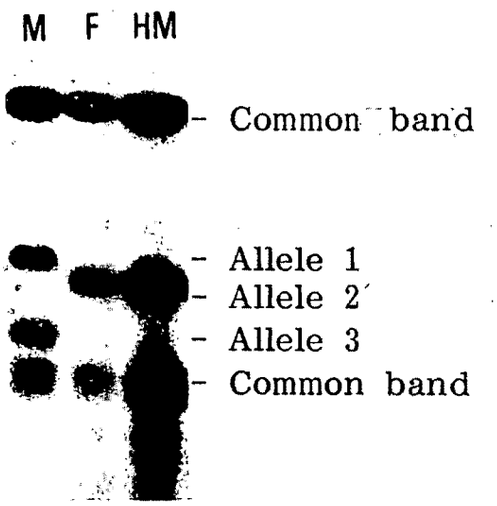


Fig. 5. Autoradiograph of DNA digestion with *Taq* I and hybridization with probe St14 in family 7. Abbreviations as in Fig. 1 legend.

dropic change of villi, which mimicks complete hydatidiform mole and blighted ovum, both maternal and paternal inheritance were demonstrated in seven of the eight cases that were collected. In case 18 with 45,X, the only X chromosome was from the paternal side, as revealed by the St14 probe. However, in case 17 with a diagnosis of blighted ovum and a histologic confirmation of chorionic villi, only paternal genetic materials were found as shown by the β-globin gene and 3′α-HVR probes (Table II). Interestingly, the cytogenetic study of the chorionic villi revealed balanced translocation between chromosomes 13 and 14, whereas the karyotypes from the peripheral blood of both parents were normal. Because of the translocation, the androgenetic mechanism does not seem to arise from duplication of a haploid sperm. Whatever the cause, the outcome of the conception seemed to be influenced by an interaction of the chromosomal translocation and the androgenetic process. With an earlier diagnosis and termination of pregnancy wastage because of the widespread use of ultrasonic scanning in early gestation, it is possible that some conceptions that are destined to become hydatidiform moles can be terminated before typical manifestations develop.

In conclusion, the restriction fragment length polymorphism analysis of complete hydatidiform mole and of the parents confirms that most cases of complete hydatidiform mole are caused by the fertilization of an empty egg by a haploid sperm, which duplicates to restore a diploid karyotype. However, in rare instances, complete hydatidiform mole may have maternal genetic contribution, and in some pregnancy wastages, pure adrogenetic inheritance may be found.

The β-globin gene, 3′α-HVR, J-Bir, St14, and pY3.4 probes were provided by Drs. Tom Maniatis, D.R. Higgs, L.M. Kunkel, J.L. Mandel, and Y.W. Kan, respectively. The EJ 6.6 clone was from American Type Culture Collection (ATCC 41028). We are grateful to Dr. Chun-Ying Chang for referral of some of the patients, to Prof. Hsu-Wen How, Department of Pathology of the University, for histologic examination, and to Prof. Ding-Shinn Chen, Graduate Institute of Clinical Medicine of the University, for various forms of assistance.

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Transplacental, amniotic, urinary, and fetal fluid dynamics during very-large-volume fetal intravenous infusions

Robert A. Brace, PhD, and Thomas R. Moore, MD
La Jolla, California

With rapid intravenous infusion of very large volumes of isotonic saline solutions into the fetus, the fluid could stay within the fetal body, thereby creating hydrops fetalis, be transferred into the amniotic fluid through the fetal kidneys, thereby creating polyhydramnios, or be transferred across the placenta into the maternal circulation. This study was designed to explore these possibilities. After a 1-hour control period, 10 near-term chronically catheterized ovine fetuses were infused intravenously with 4 L (>100% of fetal weight) of either isotonic saline solution or lactated Ringer's solution over 4 hours. Fetal arterial pressure was significantly elevated by 7 mm Hg throughout the infusion ($p < 0.00001$). Venous pressure underwent a transient rise (4.8 mm Hg) at 20 minutes of infusion and remained elevated (2.7 mm Hg) during the rest of the infusion ($p < 0.00001$). Fetal urine flow increased by an average of 5.7 ± 0.4 ml/min throughout the infusion ($p < 0.00001$) and accounted for 34.1% ± 2.6% of the infused volume. Estimated fetal extracellular fluid volume increased by 17.7% ± 1.8% of the infused volume. Because fetal fluid retention, urine flow, and amniotic fluid volume changes accounted for only half of the infused fluid, the remainder of the infused volume must have crossed the placenta and entered the maternal circluation. Given the above changes in vascular pressures, this requires a filtration coefficient of the placenta 50 to 100 times the previously reported values. Thus we conclude that relatively small changes in fetal vascular pressures dramatically alter the filtration capacity of the ovine placenta and transplacental volume flow. (AM J OBSTET GYNECOL 1991;164:907-16.)

Key words: Fetus, urine flow, amniotic fluid volume, fluid balance, placental transfer

In spite of the fact that fetal fluid balance has been studied for a number of years, there presently exists little practical understanding of what factors control the amount of fluid within the fetal compartment. Thus the underlying pathophysiologic characteristics of conditions such as hydrops fetalis or polyhydramnios are largely unknown. From theoretical considerations it is clear that both hydrostatic and osmotic forces are important determinants of the rate of transplacental fluid movement, as are the placental permeability characteristics.[1-3] However, it is unclear whether any or all of these are altered when hydrops fetalis or polyhydramnios is present.

In a recent study, we gradually infused 7 L of an isotonic physiologic saline solution into the circulation of chronically catheterized ovine fetuses over 3 days.[4] Surprisingly, the fetuses did not become edematous even though the infused volume was more than twice their body weight. Only 2 L of the 7 L infused remained within the amniotic and allantoic compartments at autopsy. Thus the fetal sheep appears efficient at transferring excess water and solute to the maternal compartment over a period of days.

Presently it is not known whether the fetus is capable of significant transfer of isotonic saline solutions to the maternal compartment over shorter time intervals. The present study was designed to test this possibility. In addition, we explored whether osmotic or hydrostatic forces mediate fetal-to-maternal transfer by comparing responses to fluids of different composition, that is, 0.9% saline solution and lactated Ringer's solution.

Methods

Chronically catheterized fetal sheep were surgically prepared and maintained as described in detail elsewhere.[5-7] During aseptic surgery with animals under gas inhalation anesthesia, catheters were implanted in the fetal descending aorta, inferior vena cava, urinary bladder, and amniotic fluid, as well as in the maternal descending aorta. Experiments were conducted 5 or more days after surgery while the ewe was housed in a metabolic cart. These experiments were approved by our institution's animal subjects committee.

During the experiments, fetal arterial pressure, venous pressure, heart rate, amniotic fluid pressure, and urine flow rate were continuously recorded as previ-

From the Division of Perinatal Medicine, Department of Reproductive Medicine, University of California at San Diego.
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Reprint requests: Robert A Brace, PhD, Department of Reproductive Medicine 0802, University of California, San Diego, La Jolla, CA 92093.
6/1/26279

ously described.[8, 9] Fetal vascular pressures were referenced to amniotic fluid pressure.[8]

Each experiment began with a 60-minute control period. At the end of the control period, 4000 ml of either warmed (40° C), lactated Ringer's solution (Baxter Corp., Deerfield, Ill.; measured osmolality, Na^+, K^+, and Cl^- concentrations: 251 mOsm/kg, 125 mEq/L, 4.0 mEq/L, and 110 mEq/L, respectively; $n = 5$) or 0.9% saline solution (Baxter Corp.; measured osmolality, Na^+, K^+, and Cl^- concentrations: 289 mOsm/kg, 152 mEq/L, 0.2 mEq/L, and 149 mEq/L, respectively; $n = 5$) were infused into the fetal circulation over 4 hours (that is, at a rate of 16.7 ml/min). Termination of the infusion was followed by a 1-hour recovery period.

Samples of fetal arterial blood, urine, and amniotic fluid as well as maternal arterial blood were taken at 30 to 60 minute intervals for measurement of osmolality (Advanced Instruments, Inc., Needham Heights, Mass.; freezing point depression) and Na^+, K^+, and Cl^- concentrations (model 5 + 5 electrolyte analyzer, Nova, Waltham, Mass.). From eight animals, fetal blood was also collected and assayed for plasma arginine vasopressin and atrial natriuretic factor concentrations as previously described.[10] Fetal blood was replaced after each sample with an equal volume of heparinized maternal blood, which was collected before the experiment. Fetal and maternal blood gases and pH were measured with an automated blood gas analyzer (model 1306, Instrumentation Laboratory, Lexington, Mass.) and were corrected to fetal (39.5° C) and maternal (39° C) body temperatures.

At 60-minute intervals ultrasonography (3.5 MHz linear-array scanner, Sonoline, Japan) was used to determine fetal thoracic, abdominal, and cardiac diameters in two dimensions. For each measurement a mean was taken from triplicate determinations. Cross-sectional areas were calculated from the product of the two diameters. Ultrasonography also was used to determine the presence or absence of free fluid within the fetal abdominal, thoracic, and pericardial cavities. In preliminary experiments with a catheter inserted into these spaces, if 5 to 10 ml or more of saline solution was injected into either the abdominal or thoracic cavity, it was detectable with our ultrasonographic method.[11] Amniotic fluid volume was estimated at hourly intervals as the mean of a triplicate determination of the four-quadrant sum for the amniotic fluid index.[12] These estimates of the amount of free fluid in the fetus and amniotic fluid volume should be interpreted continuously because our preliminary studies[11, 12] were conducted in fetuses that had been euthanized.

Fetal arterial blood volume as a percent of control was calculated from fetal hematocrits as previously described.[13] The method is valid because the ovine fetus does not have a pool of releasable red blood cells such as occurs in the spleen of the adult.[13] Fetal extracellular fluid volume changes as a fraction of control values were calculated from plasma protein concentrations by dividing the mean plasma protein concentration during the control period by that at the time of each sample. This calculation is based on the assumption that the plasma proteins rapidly distribute between the plasma and interstitial spaces and is supported by our previous observation that the capillary permeability to proteins in fetal sheep averages 15 times adult values.[14] The absolute changes in extracellular fluid volume were then calculated as the product of the above fraction, fetal weight, and an assumed initial extracellular fluid volume of 320 ml/kg.[15, 16] Although we do not know the accuracy of this approximation, it most likely overestimates the actual changes in extracellular fluid volume because the infusions would directly dilute plasma proteins and wash them into the interstitial spaces.

After the experiments the animals were euthanized with 300 mg/kg pentobarbital. The fetuses were removed, towel dried, weighed, and autopsied. No correction was made in fetal weight for fluid retained within the fetus. The net effect of the latter on the extracellular fluid volumes calculated as described above would be to overestimate the initial extracellular fluid volume at the start of the experiment and thus to overestimate the extracellular fluid volume change throughout the infusion.

Not all values were measured in every animal. Fetal urine flow rates were measured in only nine of the 10 animals because a urinary bladder catheter was not placed in one animal. Ultrasonographic measurements were made in only eight of 10 animals. Within these eight animals fetal cardiac dimensions could not be determined at all time points because of shadowing caused by positioning of the fetal bones. Maternal values were monitored in only six of the 10 animals because we were not anticipating the extensive and rapid transplacental volume shift in the first few animals.

Statistical analysis. The continuously measured fetal variables are presented as 10-minuted averages. All data are presented as the mean ± SE and were analyzed for statistically significant changes with time by a two-factor analysis of variance for repeated measures, with time and animal being the two factors. To statistically compare responses during saline solution versus Ringer's infusion, a three-factor analysis of variance was used, with time, treatment, and animal being the three factors. A significant difference in the responses of the two groups occurred when a statistically significant interaction between time and treatment was present. A value of $p < 0.05$ was taken as statistically significant, and the described changes were significant at

Table I. Fetal and maternal arterial pH, blood gases, hematocrit, and plasma protein concentration during 60-minute control period

Variable	*Fetal*	*Maternal*
Arterial pH	7.345 ± 0.007	7.457 ± 0.007
Carbon dioxide tension (mm Hg)	50.9 ± 1	36.9 ± 0.9
Oxygen tension (mm Hg)	22.1 ± 0.6	103 ± 3
Hematocrit (%)	33.3 ± 0.9	29.8 ± 1.6
Plasma protein (gm/dl)	3.6 ± 0.1	6.7 ± 0.2

Data expressed as mean ± SE. $n = 10$ for fetal values, and $n = 6$ for maternal values.

Table II. Fetal and maternal osmolalities and electrolytes during 60-minute control period

Variable	*Fetal plasma*	*Maternal plasma*	*Fetal urine*	*Amniotic fluid*
Osmolality (mOsm/kg)	296.9 ± 1.0	300.2 ± 0.9	129.7 ± 7.6	241.3 ± 9.4
Sodium (mEq/L)	142.0 ± 0.6	146.7 ± 0.7	43.7 ± 5.7	101.8 ± 6.1
Potassium (mEq/L)	4.3 ± 0.1	4.1 ± 0.2	5.1 ± 1.6	6.8 ± 1.2
Chloride (mEq/L)	110.9 ± 0.7	115.1 ± 0.5	37.8 ± 4.4	90.1 ± 5.9

Data expressed as mean ± SE. $n = 10$ for fetal plasma and amniotic fluid, $n = 9$ for fetal urine, and $n = 6$ for maternal plasma.

this level unless otherwise specified. If the analysis of variance was significant, differences among means were analyzed with Fisher's least significant difference test for multiple comparisons. If the responses to the infusion of 0.9% saline solution were not significantly different from the responses to the infusion of lactated Ringer's solution, the data were combined and only the mean responses of all animals were presented. Occasionally, symbols within the figures have no visible SE bars because the SE lies within the symbol.

Results

Fetal age was 134 ± 2 (SE) days' gestation (range, 127 to 141 days; term, 145 to 150 days) on the day of experimentation. During the 60-minute control period, fetal arterial pressure was 43.4 ± 1.0 mm Hg, venous pressure was 3.4 ± 0.3 mm Hg, heart rate was 154 ± 5 beats/min, urine flow was 0.67 ± 0.07 ml/min, plasma arginine vasopressin was 1.5 ± 0.1 pg/ml, and plasma atrial natriuretic factor was 166 ± 17 pg/ml. Additional fetal and maternal values during the control period are listed in Tables I and II. These are consistent with normal values from this laboratory.

In response to the infusion, fetal arterial pressure increased significantly (mean, 7.4 mm Hg; $p < 0.00001$) and was maintained throughout the 4-hour infusion (Fig.1). During the 1-hour recovery period, arterial pressure decreased significantly to 2.9 mm Hg below control values. Although there were highly significant increases in fetal venous pressure ($p < 0.00001$), the response differed from the arterial pressure response in that a transient maximum of only 4.8 mm Hg occurred at 20 minutes, followed by a maintained increase of 2.7 mm Hg throughout the remainder of the infusion period (Fig. 1). In addition venous pressure returned to, but not below, preinfusion values during the recovery period. Fetal heart rate underwent a small transient rise with a peak of 14 ± 5 beats/min above control ($p = 0.031$) at 40 minutes and returned to values not significantly different from control within an additional 30 minutes (Fig.1). Amniotic fluid pressure increased significantly during the infusion ($p = 0.00001$), averaging 2.1 mm Hg above control during the last hour of infusion. Amniotic fluid pressure remained at this level during the recovery period. When the responses are compared in the fetuses infused with saline solution and in those receiving lactated Ringer's solution, there were no significant differences in fetal arterial, venous, or amniotic fluid pressures or heart rate.

During the infusion there were significant decreases in fetal hematocrit ($p < 0.01$) and plasma protein concentration ($p < 0.00001$), as seen in Fig. 2. During the recovery period hematocrit increased to above control values whereas plasma protein concentration remained reduced. These changes in hematocrit correspond to a 3.2% ± 0.7% increase in blood volume throughout the infusion and a 5.6% ± 1.4% reduction afterward (Fig. 2). In contrast to these small changes in blood volume, extracellular fluid volume increased by 58.0% ± 3.4% during the last hour of infusion and remained elevated by 31.2% ± 3.5% ($p < 0.00001$) at the end of the recovery period (Fig. 2). These changes in extracellular fluid volume correspond to a 17.7% ± 1.8% retention of the infused fluid within the fetal extracellular compartment at the end of the infusion and an 8.5% ± 0.9% retention at the end of the recovery period. Again there were no statistically significant differences in the

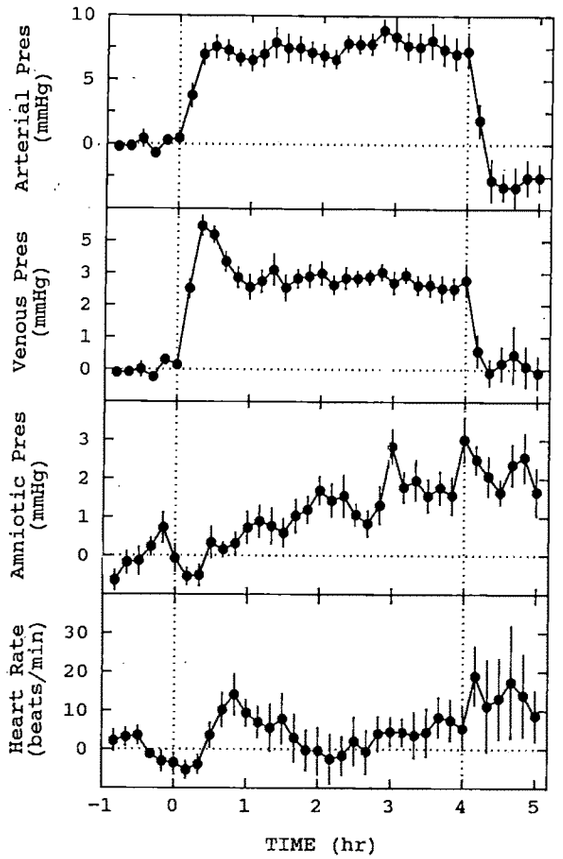


Fig. 1. Fetal arterial pressure, venous pressure, heart rate, and amniotic fluid pressure responses to infusion of 4 L of Ringer's solution or 0.9% saline solution over 4 hours. Data expressed as mean ± SE change from average value during 1-hour control period (n = 10). *Vertical dotted lines* indicate start and end of 4-hour infusion.

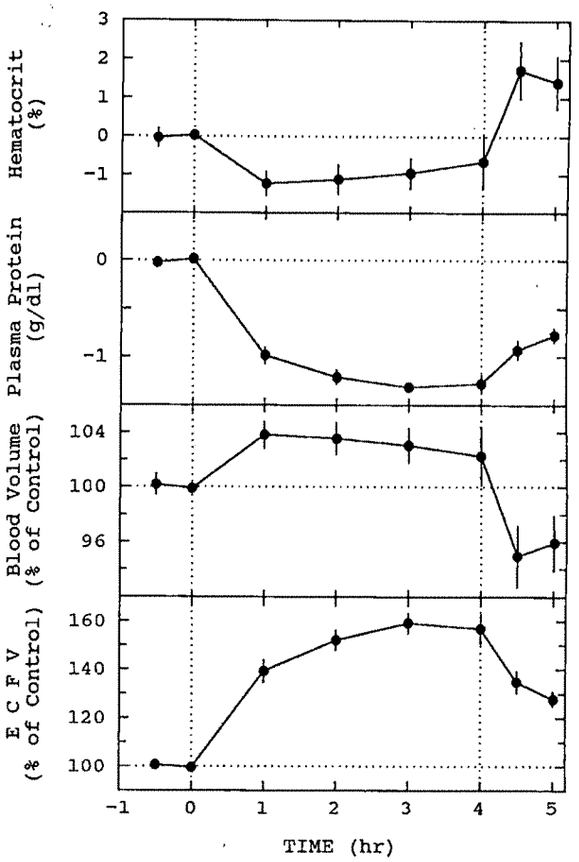


Fig. 2. Fetal hematocrit, plasma protein concentration, blood volume, and extracellular fluid volume *(ECFV)* responses to the infusion of 4 L/4 hr starting at time 0. Data are mean ± SE (n = 10) and are expressed as either a change or a percent of mean values during he control period. *Vertical dotted lines* indicate start and end of 4-hour infusion.

responses to infusion of 0.9% saline solution compared with infusion of lactated Ringer's solution.

In contrast to similar fetal pressure and volume changes, there were major differences in fetal plasma composition changes during the infusion of saline solution versus infusion of lactated Ringer's solution. As seen in Fig. 3, fetal osmolality decreased by 3.1 mOsm/kg at the end of the Ringer's infusion compared with a 7.6 mOsm/kg increase in the fetuses given saline solution (p = 0.0017, comparison of the two groups). Plasma Na^+ concentration was unchanged during Ringer's infusion and increased by 7.0 ± 0.3 mEq/L during saline solution infusion ($p < 0.00001$). Plasma Cl^- increased by 2.9 ± 1.1 mEq/L at the end of Ringer's infusion; this was significantly less ($p < 0.00001$) than the 13.9 ± 2.3 mEq/L increase during saline solution infusion (Fig. 3). Finally, although plasma K^+ concentration decreased in both groups, the decrease was much greater ($p < 0.00001$) during saline solution infusion.

The changes in maternal plasma composition were less than the fetal changes (Fig. 4). Although maternal osmolality decreased by an average of 1.8 ± 1.0 during the last 2 hours of the Ringer's infusion compared with a 1.8 ± 0.8 mOsm/kg increase during saline solution infusion, the differences were not statistically significant (p = 0.11). Maternal plasma Na^+ concentration was unchanged during Ringer's infusion and increased by 1.6 ± 0.3 mEq/L during fetal saline solution infusion; this difference was significant (p = 0.027). Maternal plasma Cl^- and K^+ were unchanged with time in either group, and there were no significant differences between the two groups in either Cl^- or K^+ responses. In addition, there were no statistically significant changes in maternal hematocrit or plasma protein concentration with time.

The changes in fetal and maternal plasma electrolytes and osmolality were accompanied by changes in the transplacental gradients in osmolality and electrolyte concentrations as seen in Fig. 5. The gradients in

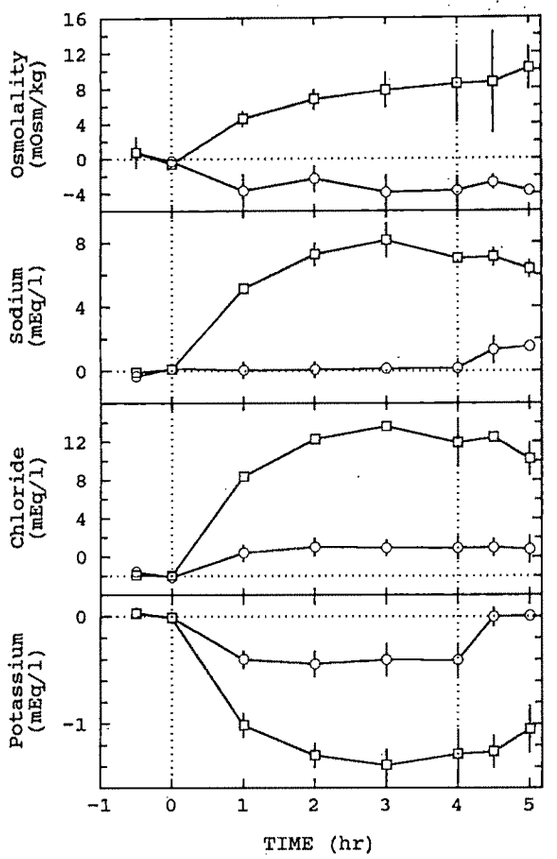


Fig. 3. Fetal plasma osmolality and electrolyte concentration changes in response to infusion of 4 L/4 hr starting at time 0. *Circles,* Ringer's infusion; *squares,* 0.9% saline solution infusion. Data expressed as mean ± SE changes from values during 1-hour preinfusion period.

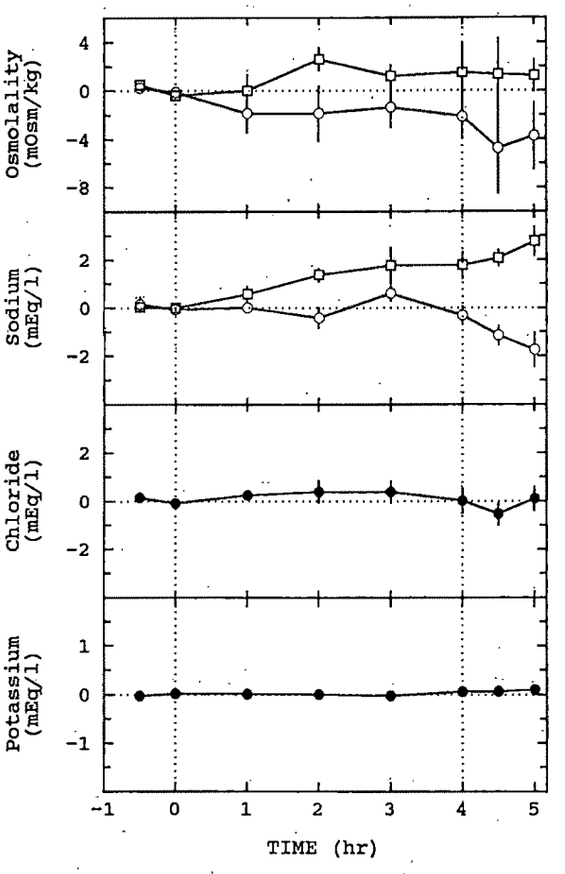


Fig. 4. Maternal plasma osmolality and electrolyte changes in response to infusion of 4 L/4 hr into fetal circulation starting at time 0. *Circles,* Ringer's infusion; *squares,* 0.9% saline solution infusion; *filled circles,* combined Ringer's plus saline solution infusion when no significant differences existed between the two groups. Data expressed as mean ± SE changes from values during 1-hour preinfusion period.

plasma osmolality, Na^+, Cl^-, and K^+ were each significantly different in animals given saline solution versus animals given lactated Ringer's solution ($p < 0.01$ for all).

Fetal urine flow rate underwent very large increases during the infusion ($p < 0.00001$), as seen in Fig. 6. Urine flow increased gradually throughout the 4-hour infusion and was 7.7 ± 0.5 ml/min above control at the end of the infusion period. On the average, urine flow increased by a mean of 5.7 ± 0.4 ml/min throughout the 4-hour infusion. This corresponds to 34.1% ± 2.6% of the infused volume. Urine flow rate decreased rapidly after termination of the infusion (Fig. 6) and was 2.3 ± 0.4 ml/min above control at the end of the 1-hour recovery period. The urine flow response was not significantly different in fetuses given saline solution versus fetuses given lactated Ringer's solution.

There were large and highly significant increases ($p < 0.00001$) in fetal urine osmolality and in Na^+ and Cl^- concentrations in response to the infusion (Fig. 7); urine K^+ concentration was unchanged. Although there were no statistically significant difference between fetuses given saline solution and fetusus given Ringer's solution, urine Cl^- tended to increase more in the fetuses given saline solution ($p = 0.108$). During the last hour of infusion the composition of fetal urine was essentially the same as that of the infusate during Ringer's infusion (mean osmolality, 255 mOsm/kg; Na^+, 121 mEq/L; K^+, 3.1 mEq/L; Cl^-, 103 mEq/L), and was slightly less concentrated as compared with the infusate during 0.9% saline infusion (mean osmolality, 269 mOsm/kg; Na^+, 132 mEq/L; K^+, 2.4 mEq/L; Cl^-, 122 mEq/L).

Although there were highly significant ($p < 0.0001$) increases in fetal plasma atrial natriuretic factor in response to the infusion (Fig. 8), the changes did not parallel those in fetal urine flow. In addition, there was a rise in the mean plasma arginine vasopressin concen-

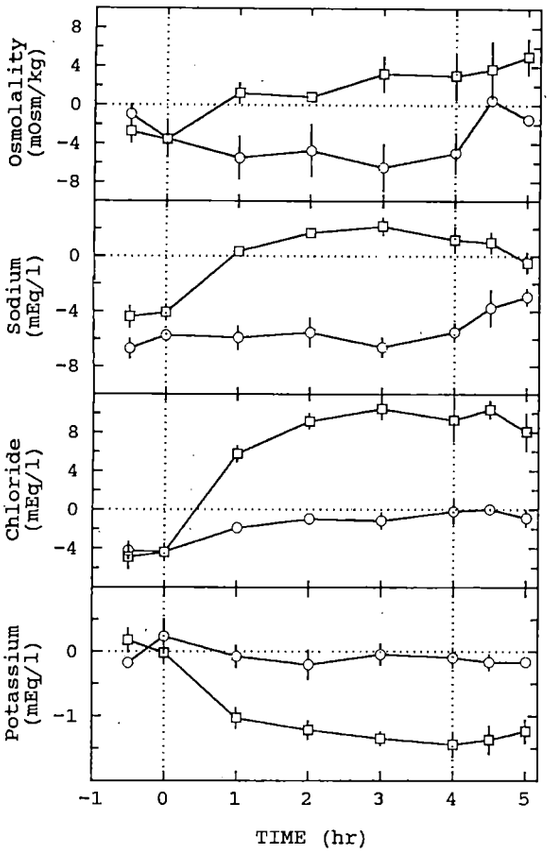


Fig. 5. Changes in transplacental gradients in plasma osmolality and electrolyte concentrations in response to infusion of 1 L/hr from 0 to 4 hours. *Circles,* Ringer's infusion; *squares,* 0.9% saline solution infusion. Data expressed as mean ± SE changes from values during 1-hour preinfusion period.

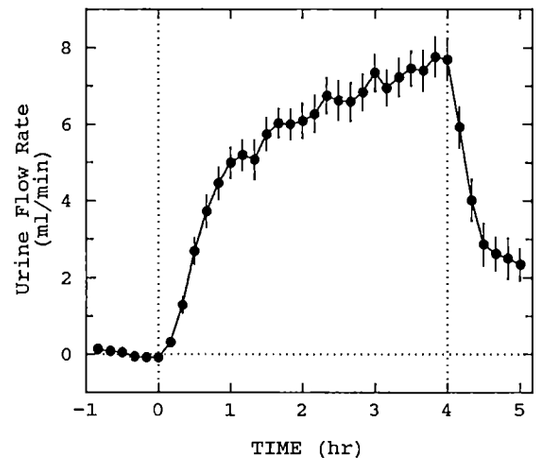


Fig. 6. Fetal urine flow rate responses to infusion of 1 L/hr of Ringer's solution and saline solution into fetal circulation from time 0 to 4 hours. Data expressed as mean ± SE changes from values during 1-hour control period.

tration (Fig. 8), but this was not significant because arginine vasopressin was unchanged in half of the fetuses and rose in the other half.

There were multiple changes in amniotic fluid composition during the infusion (Fig. 9). Amniotic fluid osmolality ($p = 0.0021$), Na^+ concentration ($p = 0.0001$), and Cl^- concentration ($p = 0.0004$) increased significantly, and there were no differences in the fetuses given saline solution versus those given Ringer's solution. In contrast, the decrease in amniotic K^+ concentration was significantly greater ($p = 0.0008$) in the fetuses given saline solution (Fig. 9).

Fetal arterial oxygen and carbon dioxide tensions were unchanged in response to the infusion (Fig. 10). Fetal pH decreased significantly during both infusions ($p < 0.00001$), but the decrease was significantly greater during saline solution infusion ($p < 0.030$). Maternal blood gas tensions and pH were unchanged in both groups, and there were no significant differences between groups in the maternal values.

As determined ultrasonographically, neither fetal thoracic nor pericardial cross-sectional area changed significantly during the infusion (Fig. 11). Fetal abdominal area increased by 41.1% ± 12.7% ($p < 0.01$) and remained elevated during the recovery period (Fig. 11). No free fluid was detected within the thoracic cavity in any of the fetuses. In addition, even with the increase in abdominal area, we detected no free fluid in four of eight fetuses; minimal ascites was observed in two, and mild ascites was seen in the remaining two fetuses. In half of the fetuses we observed with ultrasonographic examination a notable increase in the volume of fluid within the rumen. The amniotic fluid index averaged 105 ± 21 mm during the control period; it increased by 191% ± 59% ($p < 0.001$) at the end of the infusion period (Fig. 11.)

Fetal weight at autopsy was 3.95 ± 0.32 kg. None of the fetuses were hydropic at autopsy. This fetal weight is slightly, but not significantly, greater than that in other fetuses studied in this laboratory at the same gestational age,[4] but not subject to rapid infusions.

Comment

In this study we intravascularly infused the ovine fetus at a rate of 16.7 ml/min for 4 hours with physiologic saline solutions so the total infused volume was 4000 ml. Because this volume was in excess of fetal weight, we anticipated that the fetuses would become severely edematous. This expectation of fetal edema was based on a series of previous studies in the fetal sheep:

1. In response to acute vascular volume loading of smaller magnitudes, the maximal increase in fetal urine flow was <1 ml/min,[9] so that only a very small fraction of the infused fluid was expected

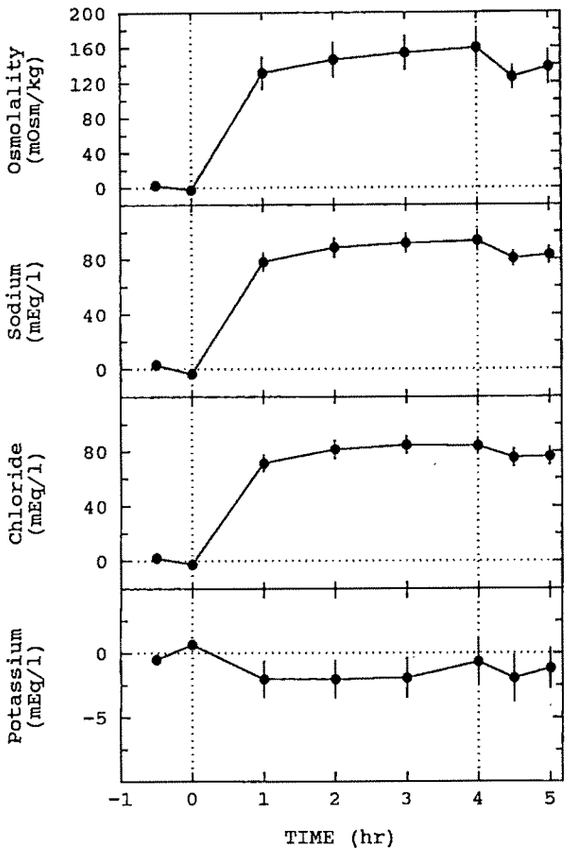


Fig. 7. Changes in fetal urine osmolality and electrolyte concentrations in response to infusion of 1 L/hr of Ringer's solution or saline solution into fetal circulation from time 0 to 4 hours. Data expressed as mean ± SE from values during 1-hour control period.

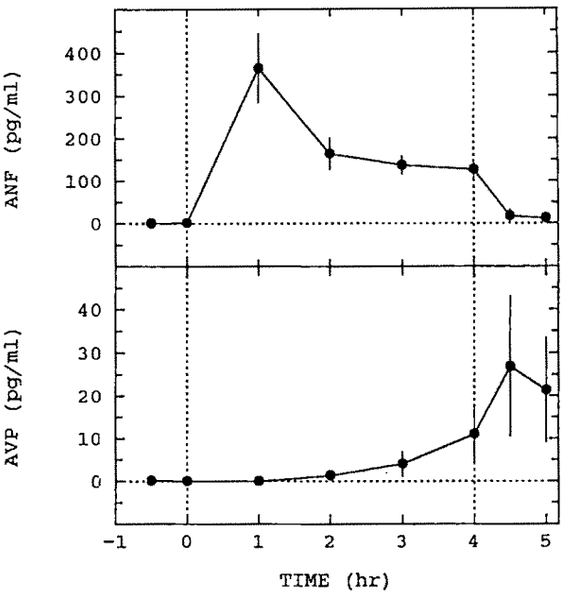


Fig. 8. Mean ± SE fetal plasma atrial natriuretic factor *(ANF)* and arginine vasopressin *(AVP)* responses to the infusion of 4 L/4 hr of fluid into the fetal circulation. Data expressed as changes from mean values during 1-hour control period.

to be excreted through the fetal kidneys. Even with long-term volume loading over a period of days, the maximal increase in fetal urine flow was 5 ml/min,[4] again suggesting only a small fraction of the fluids infused in the present study would be excreted by the kidneys.

2. The placental filtration coefficient in the chronically catheterized sheep has been reported to be 0.0067 ml/min/kg of fetal weight[17] or roughly 0.025 ml/min for fetuses with an anticipated weight of 3.5 kg. With this filtration coefficient, an increase in fetal vascular pressures of 667 mm Hg would be required to hydrostatically drive the infused 16.7 ml/min across the placenta. This is not likely in view of the normal arterial and venous pressures of 45 and 3 mm Hg, respectively, in the fetuses.
3. Although the late-gestation fetus normally secretes roughly 0.2 ml/min of fluid from its lungs, the lung secretion rate decreases with vascular volume loading.[18] Thus, by considering each of the major routes by which the infused fluid might leave the fetus, we speculated that a large fraction of the 4000 ml would remain within the fetal body at the end of the 4-hour infusion.

In contrast to this expectation, we observed that little of the infused fluid remained within the fetal body at the end of the infusion. This conclusion is based on two indirect estimates of extracellular fluid volume changes. First, we used the plasma proteins as an extracellular marker to calculate extracellular fluid volume changes. We acknowledge that this is probably a crude marker at best that, as considered in the Methods section above, probably leads to an overestimation of fetal extracellular fluid volume changes for at least two reasons. Even with the overestimations, we found only 17.7% of the infused fluid was retained within the fetal body. Second, we used ultrasonography to monitor fetal cross-sectional areas and to monitor free fluid within the thoracic and abdominal cavities. Although thoracic and pericardial areas were unchanged, abdominal area increased significantly. Because we have previously found that 5 to 10 ml of fluid injected directly into the thoracic or abdominal cavities of fetal sheep could be detected ultrasonographically,[11] our ultrasonographic monitoring suggested that only small volumes of fluid were retained within the fetal body. In addition to these two indirect estimates, fetal blood volume increased by only 3.5% during the infusion. Thus it is clear that only a small fraction of the infused fluid remained within the fetal body at the end of the infusion.

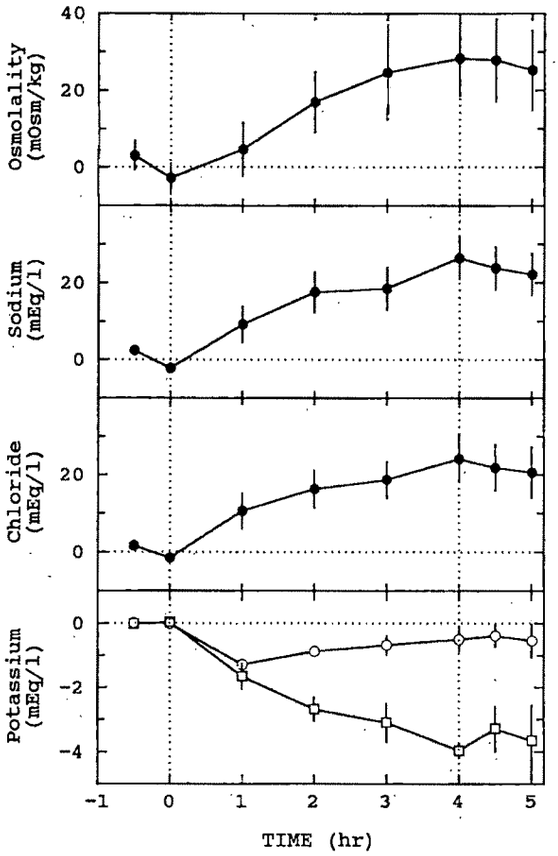


Fig. 9. Amniotic fluid osmolality and electrolyte concentration changes in response to intravascular infusion of 4 L of Ringer's solution or 0.9% saline solution into fetus from 0 to 4 hours. Data expressed as mean ± SE. *Circles,* Ringer's infusion; *squares,* 0.9% saline solution infusion; *filled circles,* combined Ringer's plus saline solution infusion when no significant differences existed between the two groups.

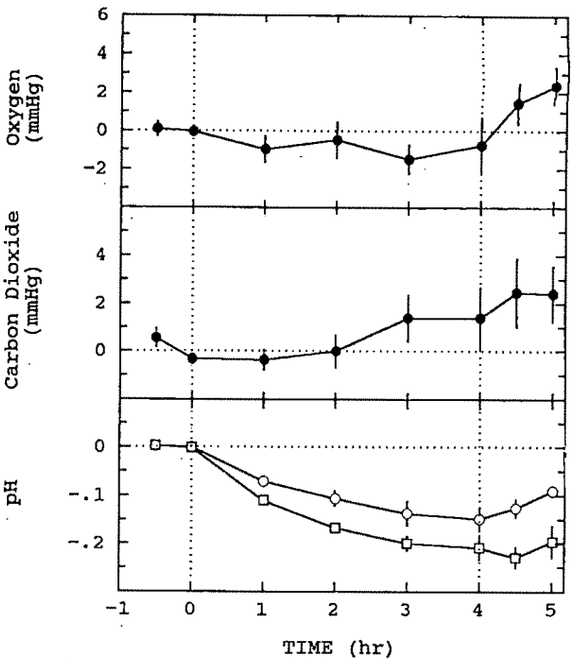


Fig. 10. Changes in fetal arterial blood gases and pH during the infusion of 4 L of Ringer's solution or saline solution into fetal circulation. Infusion was from time 0 to 4 hours. *Circles,* Ringer's infusion; *squares,* 0.9% saline solution infusion; *filled circles,* combined Ringer's plus saline solution infusion when no significant differences existed between the two groups. Data expressed as mean ± SE changes from values during 1-hour preinfusion period.

The fetal urine flow response to the infusion of 16.7 ml/min is remarkable in that the flow of 7.4 ml/min above baseline values during the last hour of infusion is by far the highest urine flow rate ever reported for the ovine fetus. In spite of this high urine flow, excretion by the fetal kidneys accounted for only 34.1% of the infused volume over the 4 hours. In addition, the change in amniotic plus allantoic fluid volume, as estimated from our previous study of the amniotic fluid index in pregnant sheep[12] agrees well with that anticipated from the measured changes in fetal urine flow. Finally, the changes in amniotic composition that occurred (Fig. 9) are those predicted if 1.4 L (i.e., 34.1% of 4 L) of urine of the measured composition was added to 1 L of amniotic fluid.

By combining the fluid excreted by the fetal kidneys with that retained within the fetal body, we could account for only 50% of the infused fluid. It is clear that the remaining 50% of the infused volume, or 2000 ml, must have crossed the placenta and entered the maternal circulation during the 4-hour infusion. Because the mean increase in arterial and venous pressures combined was only 5.2 mm Hg during the infusion, the filtration coefficient of the placenta would equal 2000 ml/240 min/5.2 mm Hg or 1.60 ml/min/mm Hg. This number is 64 times the value of 0.025 ml/min expected for fetuses weighing 3.5 kg. Thus it appears that there was a 50-fold to 100-fold increase in the filtration coefficient of the placenta during the infusion. This is remarkable because it occurred with a mean increase in venous pressure of <3 mm Hg and in arterial pressure of 7 mm Hg. These pressure increases are comparable to those that occur during acute fetal hypoxia[19, 20] and are less than those that occur during spontaneous fetal body movement. Thus, although it may be suggested that the results of this study are not physiologically relevant because of the large volumes of fluid that were administered, this argument appears invalid because the pressure changes that occurred in the fetus are well within the physiologic range.

It might also be suggested that the large rise in the placental filtration coefficient that occurred in this study was hormonally mediated. Although this is a pos-

sibility, we presently tend to discount the possibility because fetal arginine vasopressin was not consistently changed. Additionally, although fetal atrial natriuretic factor rose during the last 3 hours of the infusion, atrial natriuretic factor was only twice normal values. In view of previous studies in which exogenous atrial natriuretic factor was infused into the ovine fetus,[21] it is unlikely that the small rise in atrial natriuretic factor mediated the huge increase in placental permeability. Thus it appears that the small rise in fetal vascular pressures may have mediated the placental permeability rise.

In addition to the marked increase in the placental filtration coefficient, there appear to be major changes in the reflection coefficients of the placenta. Previously the reflection coefficients of the ovine placenta for Na^+ and Cl^- have been estimated to be approximately 0.8.[22] Considering that the changes in fetal electrolyte composition were either nonexistent or small and that urine was nearly isotonic with the infusate, it is clear that the composition of the fluid crossing the placenta must have been nearly the same as the infusate. This would require the placental reflection coefficients to be near zero for both Na^+ and Cl^-. Because these are drastically different from the value of 0.8 noted here, our observations further suggest that there were major increases in the placental permeability during the 4 L infusions. Again, a remarkable aspect of the increased permeability is that such large changes occurred in the face of only small changes in fetal vascular pressures.

There is an alternative interpretation to these data, that is, the sheep placenta normally has a high filtration coefficient and low reflection coefficients, and previous estimates of these placental permeability parameters simply may have been in error. Such errors might occur if the simplified theory used to calculate permeability failed to adequately represent the complex exchange processes within the placenta, if there are inhomogeneous permeabilities and exchanges within the placenta, or if the high osmolalities used in calculating the filtration coefficient altered the permeability characteristics of the placenta. This possibility is supported by two observations: (1) Very large volumes of fluid with Na^+ and Cl^- content many times that in the fetal body can be drained as either amniotic fluid or fetal urine over a period of many days without any indication of electrolyte or osmolality changes within the fetus[5, 22, 23]; (2) 5 L of fluid and electrolytes was transferred from the fetal to the maternal compartment during a 3-day intravascular infusion into the fetus without changes in fetal or maternal plasma composition.[4] Although the latter two observations appear to contradict the concept that the sheep placenta is a low-permeability organ, it is possible that the permeability was increased under the conditions of the volume loading and drainage experiments but the causes of these changes would be unknown. This possibility is supported by the study of Thornburg et al.,[22] in which it was suggested that arginine vasopressin may affect ovine placental permeability. It is noteworthy that, if fetal vascular pressures were to change, the changes most likely would be in the opposite direction during the drainage as compared with volume-loading experiments, so that the role of hydrostatic pressure changes under the two conditions to any adaptive changes in placental permeability would likely be in opposite directions.

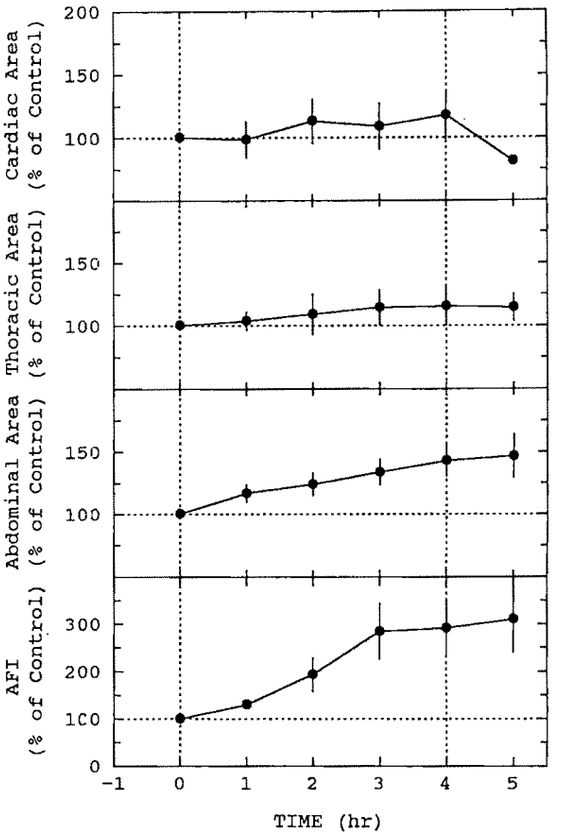


Fig. 11. Responses of fetal cardiac, thoracic, and abdominal areas and amniotic fluid index *(AFI)* to intravascular infusion of 4 L/4 hr of fluid into fetal circulation. Data expressed as mean ± SE percent of values during 1-hour preinfusion period.

In this study amniotic fluid pressure increased by 2 mm Hg at the end of the infusion period. In a recent study[24] we found that the compliance of the uterine compartment in pregnant sheep during late gestation was 1 mm Hg/L. Thus, from the increase in amniotic fluid pressure, an increase in uterine volume of 2 L would be expected in this study. This agrees with the 2 L of fluid that was estimated to be retained within the fetal body plus that excreted by the fetal kidneys

into the amniotic fluid. The latter further supports our conclusion that 2 L of fluid was transferred to the maternal compartment during this experiment. In addition, the observed changes in maternal plasma Na^+ and osmolality (Fig. 5) further support the fetal-to-maternal transfer of fluid.

Although there were several statistically significant differences in fetal plasma osmolality and electrolyte changes during the Ringer's infusion versus 0.9% saline solution infusion, there were no differences in fetal blood volume, extracellular fluid volume, or urine flow rate changes. In addition, there were no differences in the fetal vascular pressure changes between the two groups. This suggests that the large increases in placental permeability that occurred during the infusions were independent of the compositional changes in the fetus. Thus the hydrostatic pressure changes may be the primary mediators of the increased placental permeabilities.

In summary, our study shows that if huge amounts of isotonic electrolyte solutions are rapidly infused into the ovine fetus, only a small amount of fluid is retained within the fetal body and one third of the fluid is excreted through the fetal kidneys. The remainder, or roughly half, of the infused fluid appears to cross the placenta into the maternal circulation. The latter appears to be driven by surprisingly small increases in fetal vascular pressures, which produce very large increases in the placental filtration coefficient and major reductions in the placental reflection coefficients.

We sincerely appreciate the technical assistance of Sandra Miscovich, Michael Ternavan, Eddie Kwan, and Wendy Ewing.

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Enhancement of germ tube formation in *Candida albicans* by β-endorphin

Steven S. Witkin, PhD, and Aliza Kalo-Klein, PhD
New York, New York

Candida albicans is a dimorphic yeast that causes vaginal infections after its transition from a budding yeast to a germinating hyphal form. We report here that physiologic concentrations of β-endorphin, a neuropeptide with immunomodulating activity produced during stress or physical exercise, stimulates germ tube formation in *C. albicans*. The percent of germination was proportional to the endorphin concentration, over the 5×10^{-12} to 5×10^{-10} mol/L range tested. β-Endorphin modified by removal of the 4-carboxy-terminal amino acids and (D-Ala^2)-β-endorphin, a peptide with a protease-resistant amino terminal end, were equally effective in stimulating germination. In contrast, *N*-acetylated β-endorphin did not stimulate germination. Antisera to β-endorphin also completely blocked β-endorphin-stimulated germ tube formation. Two clinical isolates of *C. albicans* were also responsive to β-endorphin. Stimulation of germ tube formation by β-endorphin occurred only in sera from ovulating women. Germination in sera from women using oral contraceptives, in sera from men, or in glucose beef extract broth was not influenced by β-endorphin. Thus *C. albicans* may be able to recognize and respond to neuroendocrine signals in ovulating women. (Am J Obstet Gynecol 1991;164:917-20.)

Key words: β-Endorphin, *Candida albicans*, vaginitis, stress

Candida albicans is a dimorphic yeast that resides on vaginal mucosal surfaces in approximately 20% of women without initiating any signs or symptoms of clinical disease.[1] The proliferation of the organism is regulated principally, if not entirely, by cell-mediated immune mechanisms.[2] Therefore under conditions of depressed cell-mediated immunity *C. albicans* can increase in concentration and initiate clinical disease at mucosal sites.[1] One factor associated with the change of *C. albicans* from a commensal to a pathogenic organism is its conversion from the yeast form to a germinated hyphal form. Although the yeast form can also be associated with disease, the germinated form is better able to penetrate mucosal epithelia and induce clinical infections.[3, 4]

We have been examining the relationship between immunosuppression, germ tube formation, and vaginal mucosal infection by *C. albicans*. Women with recurrent candidal vaginitis were shown to be deficient in their in vitro cell-mediated response to *Candida* antigens.[2, 5-7] The mechanism appeared to involve prostaglandin-mediated immunosuppression. The addition of prostaglandin inhibitors to cultures of patients' peripheral blood mononuclear cells resulted in a normal proliferative response to *Candida*.[7] Furthermore, vaginal fluids of patients, but not controls, contained prostaglandin E_2 (PGE_2).[8] PGE_2 is a macrophage product that induces immunosuppression by inhibiting T-cell activation.[9] Recent studies have shown that PGE_2 also directly stimulates germ tube formation in *C. albicans*.[10] Conversely, interferon gamma, a T-cell activation product,[11] inhibits germ tube formation, even in the presence of PGE_2.[10] Thus, when cell-mediated immunity is transiently compromised through a PGE_2-mediated mechanism, *C. albicans* proliferates and is stimulated to undergo a transition from the yeast to the germ tube form. These events increase the likelihood for initiating a symptomatic vaginal infection.

In this communication, we report that *C. albicans* is also responsive to a neuropeptide, β-endorphin, that possesses immunomodulatory activity. β-Endorphin is produced by the anterior pituitary gland; its level in the circulation increases during stress and strenuous physical exercise.[12] Although the immune consequences of β-endorphin–immune system interaction need further clarification, receptors for β-endorphin have been identified on macrophages[13] and T lymphocytes.[14] It appears that the binding of β-endorphin to macrophages induces PGE_2 production that subsequently inhibits interferon gamma synthesis.[13] We demonstrate here that β-endorphin stimulates germ tube formation in *C. albicans*.

From the Immunology Division, Department of Obstetrics and Gynecology, Cornell University Medical College.
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Reprint requests: Steven S. Witkin, PhD, Dept. of Obstetrics and Gynecology, Cornell University Medical College, 525 E. 68th St., New York, NY 10021.
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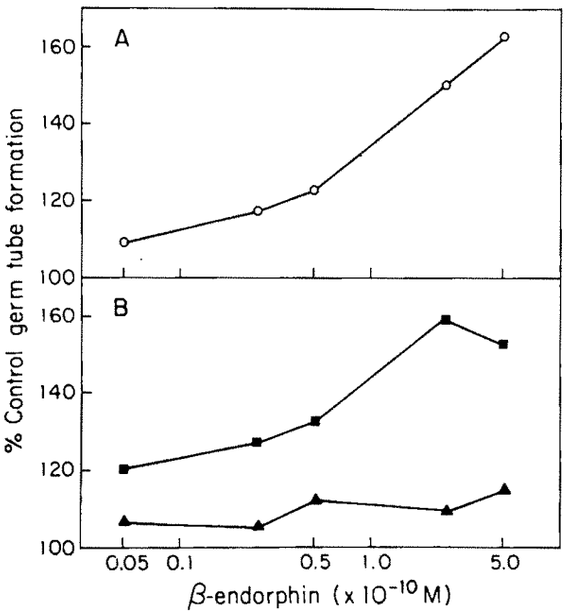


Fig. 1. Induction of germ tube formation in *C. albicans* by β-endorphin. *Panel A*, *C. albicans* yeast forms were incubated in human serum containing various concentrations of β-endorphin (○). *Panel B*, *C. albicans* was incubated in serum containing *N*-acetylated β-endorphin (▲) or carboxy-terminal–deleted β-endorphin (■). Quadruplicate aliquots were removed after 120 minutes; 400 cells were scored for yeast forms or germinated forms, and the percent germination relative to control was determined.

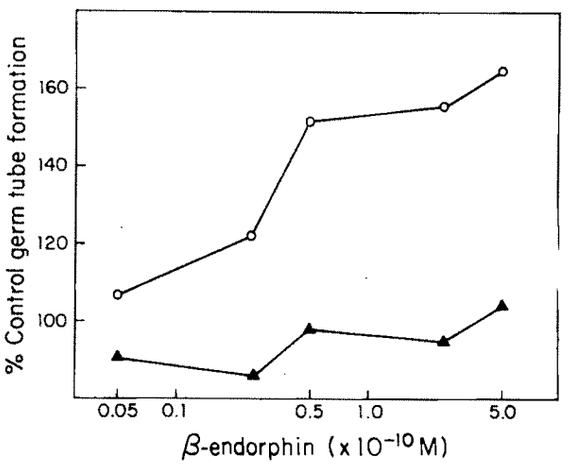


Fig. 2. Effect of antiserum to β-endorphin on germ tube formation in *C. albicans* induced by β-endorphin. Various concentrations of β-endorphin were incubated for 60 minutes at 37° C in the presence of 1 : 10 dilution of either rabbit antisera to β-endorphin (▲) or control rabbit serum (○). *C. albicans* was then added, and germ tube formation was assessed after 120 minutes, as described in Fig. 1.

Table I. Effect of β-endorphin on germ tube formation in different isolates of *C. albicans**

Isolate	*β-Endorphin (mol/L)*	*% Control germination*
Strain 925	2.5×10^{-10}	156.3
	5.0×10^{-11}	146.1
Isolate 10	2.5×10^{-10}	160.4
	5.0×10^{-11}	149.0
Isolate 6	2.5×10^{-10}	152.0
	5.0×10^{-11}	150.1

*Three isolates of *C. albicans* were incubated in human serum in the presence or absence of β-endorphin. After 120 minutes, 400 cells were counted in quadruplicate and the numbers of yeast forms and germinated forms were determined.

Material and methods

Organisms. Three isolates of *C. albicans* were used in these studies. Strain 925, our laboratory standard, was initially purchased from Presque Isle Cultures (Presque Isle, Pa.). Two clinical isolates, No. 6 and No. 10, were cultured from vaginal fluid of two women with *Candida* vaginitis. The organisms were maintained on Sabouraud's agar slants at 28° C in a 5% carbon dioxide–humidified atmosphere. Under these conditions, 100% of the cells were in the yeast form. For all experiments colonies were harvested from 24-hour cultures and suspended in phosphate-buffered saline solution; concentrations were determined with a hemacytometer. Aliquots were then centrifuged and resuspended in the desired medium.

Chemicals. β-Endorphin, *N*-acetylated β-endorphin, β-endorphin lacking the 4-carboxy terminal amino acids, and (D-Ala²)-β-endorphin were obtained from Peninsula Laboratories (Belmont, Calif.). The compounds were suspended in phosphate-buffered saline solution and diluted to the desired concentration in serum.

Sera. Blood was obtained by venipuncture from healthy men and women. After clot formation, serum was collected by centrifugation, heat inactivated at 56° C for 30 minutes, and stored in aliquots at −70° C until used. Rabbit antiserum to β-endorphin was obtained from Peninsula Laboratories; sera from unimmunized rabbits were obtained from Pel Freeze Biologicals (Rogers, Ark.).

Germ tube formation. Yeast cells were suspended at a concentration of 10^8 ml in serum, and 0.1 ml aliquots were added to an equal volume of serum containing the desired concentration of β-endorphin or β-endorphin derivative. Control samples contained no additions. After 120 minutes at 37° C, 25 μl aliquots were removed in quadruplicate, and 400 cells were scored under ×200 power for yeast and germinating forms. In all experiments replicate aliquots differed by <15%. In nine separate experiments 29.0% ± 2.0% of the strain 925 yeast forms underwent germination under these conditions, in the absence of any additions.

Table II. Ability of β-endorphin to stimulate *Candida* germ tube formation in different sera*

Subject No.	Sex	Oral contraceptive usage	% Control germination	
			5.0×10^{-10} mol/L†	2.5×10^{-11} mol/L†
1	F	No	176.0	165.0
2	F	No	169.9	163.0
3	F	No	137.0	138.0
4	F	No	132.0	123.0
5	F	Yes	106.0	95.0
6	F	Yes	99.0	104.0
7	F	Yes	109.0	99.0
8	M	—	88.0	89.6
9	M	—	101.6	107.4
10	M	—	105.0	ND

ND, Not determined.

**C. albicans* was incubated in sera from different donors in the presence or absence of β-endorphin. After 120 minutes, the percentage of yeast forms undergoing germ tube formation were determined as described in Table I.

†Concentrations of β-endorphin.

Results

Effect of β-endorphin on germ tube formation. Physiologic concentrations of β-endorphin, 5×10^{-10} to 5×10^{-12} mol/L, were tested for their effect on germ tube formation in serum, with strain 925 of *C. albicans*. As shown in Fig. 1, *A*, germ tube formation was increasingly stimulated by β-endorphin over the range of concentrations tested. At 5×10^{-10} mol/L, germ tube formation was 160% of the level found in control samples.

Effect of *N*-terminal or carboxy-terminal modifications on ability of β-endorphin to stimulate germ tube formation. The effects of *N*-acetylated β-endorphin and carboxy-terminal–deleted β-endorphin on germ tube formation are shown in Fig. 1, *B*. Blocking the *N*-terminal of β-endorphin completely obviated its ability to affect germ tube formation. In marked contrast, deletion of the carboxy-terminal had no effect; stimulation of germ tube formation occurred to the same extent as with unmodified β-endorphin. These results suggested that the interaction of β-endorphin with *C. albicans* may be receptor mediated. β-Endorphin binds to opiate receptors by its *N*-terminal; *N*-acetylation completely blocks this binding.[15] β-Endorphin binding to macrophages occurs through a similar mechanism.[13] Conversely, β-endorphin binding to lymphocytes occurs through the carboxy-terminal end.[14]

Germ tube formation in *C. albicans* can be stimulated by free amino acids.[16] To determine whether this might be the mechanism of β-endorphin stimulation of germination, the ability of (D-Ala²)-β-endorphin to promote germ tube formation was evaluated. (D-Ala²)-β-endorphin is a modified endorphin whose *N*-terminal is resistant to proteolysis. Germ tube formation was stimulated by a mean of 128.3% with 2.5×10^{-12} mol/L and 181.5% with 5.0×10^{-11} mol/L D-Ala²-β-endorphin. This result strongly suggested that intact β-endorphin, and not its degradation products, was promoting germ tube formation.

Effect of antiserum to β-endorphin on germination. To further assess the ability of β-endorphin to promote *Candida* germination, the effect of antiserum to β-endorphin was evaluated (Fig. 2). Preincubation of β-endorphin in a 1:10 dilution of specific antiserum to β-endorphin completely obliterated its ability to influence germination; parallel incubation of β-endorphin in control rabbit sera had no effect on the stimulation of germ tube formation by β-endorphin.

β-Endorphin–induced germination in clinical isolates of *C. albicans*. To assess whether stimulation of germ tube formation of *C. albicans* by β-endorphin was a general phenomenon, two recent clinical isolates of *C. albicans* were also tested. The results (Table I) clearly demonstrated that β-endorphin was equally effective in stimulating germ tube formation in isolates from clinical material and in a laboratory strain.

Effect of serum donor on β-endorphin–induced germ tube formation. All of these experiments were performed with serum from one female donor. Germ tube formation in *C. albicans* also readily occurs in glucose beef extract broth.[10] To our surprise, β-endorphin at concentrations of 5×10^{-8} to 5×10^{-12} mol/L had no effect on germ tube formation in this medium (data not shown). It appeared therefore that a serum component might be an essential cofactor for β-endorphin–stimulated germination. To further evaluate this possibility, the ability of sera from different donors to support β-endorphin–stimulated germination was examined (Table II). In the absence of β-endorphin, all

of the sera induced germ tube formation to a similar extent. Addition of β-endorphin to sera from four premenopausal women not using oral contraceptives led to a further 32% to 76% stimulation of germination. In marked contrast, β-endorphin had no effect on germination in sera from three men and in sera from three premenopausal women who were taking oral contraceptives.

Comment

Premenopausal women constitute the group at greatest risk for *Candida* vaginitis.[1] β-Endorphin, at physiological concentrations, stimulated germ tube formation in sera from this population. Circulating β-endorphin, in common with other serum proteins, undoubtedly enters the vagina through transduction and thus can affect *Candida* residing on mucosal surfaces. β-Endorphin has, in fact, been identified in uterine fluid.[17] Thus, in ovulating women harboring *C. albicans* as a commensal organism, the induction of β-endorphin by stress or physical exertion may directly increase the probability of contracting *Candida* vaginitis.

Stimulation of *Candida* germination by β-endorphin occurred only in sera from ovulating women. A circulating component unique to these women might therefore be an essential cofactor for the use or recognition of β-endorphin by *C. albicans*. Preliminary findings indicated that the extent of β-endorphin stimulation of germ tube formation was proportional to the estradiol concentration of the serum sample (unpublished data). *C. albicans* can bind progesterone[18] and estradiol[19] by receptor-like mechanisms.

Our data suggest that β-endorphin influences germination by a hormonal effect rather than as a nutritional substrate. Whether *C. albicans* possesses a receptor for β-endorphin remains to be established. However, similar to the mechanism of β-endorphin binding to opiate receptors,[15] we demonstrated that an intact *N*-terminal was required for the stimulation of germ tubes by β-endorphin.

Coupled with our previous findings, these results suggest that there is direct communication between the nervous system, the immune system, the endocrine system, and a unicellular eukaryotic microorganism. *C. albicans* has the ability to determine, through the presence of PGE_2 or β-endorphin, or the absence of interferon gamma, when there is a localized down-regulation of cell-mediated immunity. Under these conditions, when defenses against *C. albicans* are reduced, PGE_2 and β-endorphin also directly stimulate the yeast form of the organism to undergo morphogenesis and become capable of invading the mucosal epithelia. Development by *C. albicans* of this ability to "sense" the immune, neurologic, and endocrine status of the mammalian host may contribute to its survival. Methods to inhibit PGE_2 or β-endorphin production may ultimately prove to be of value in limiting the recurrences of *Candida* infection in susceptible individuals.

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The effect of volume expansion on placental blood flow in awake hypovolemic rats in late pregnancy

Peter F. Boekkooi, MD, Carla M. Verkeste, BSc, and Louis L.H. Peeters, MD, PhD
Maastricht, The Netherlands

Symptomatic hypovolemia in preeclampsia is often treated by volume expansion. However, the effect of this treatment on placental blood flow is unclear. In the present study the hypothesis was tested that slow volume expansion, imposed on volume-depleted rats, does not compromise placental blood flow. Mild hypovolemia was induced in rats in late pregnancy by 24 hours' thirsting. The resulting gradual dehydration was associated with a 7% reduction in blood volume. These rats were then subjected to blood volume expansion by a continuous infusion with a plasma substitute. Volume expansion increased blood volume by 32% and cardiac output by 73%. The extra cardiac output was distributed to kidneys, carcass, and portal bed. Placental blood flow decreased by 31%. The pattern of response was comparable to the one previously observed in normovolemic rats. However, the magnitude of the changes was larger, probably associated with a delayed or impaired diuretic response. These data suggest that volume expansion therapy in midly hypovolemic pregnant rats elicits an exaggerated hemodynamic response as compared with normovolemic rats, including placental compromise. (AM J OBSTET GYNECOL 1991;164:921-6.)

Key words: Pregnancy, rat blood volume, cardiac output, microspheres, placental blood flow, preeclampsia

The only causal treatment for preeclampsia is delivery of the fetus. However, when the gestational age is less than ≈ 30 weeks, the fetal risks associated with premature birth may be higher than those associated with preeclampsia. In recent years, symptomatic treatment by volume expansion with plasma or plasma substitutes has rapidly gained popularity. The objective of this therapy is to "correct" the contracted plasma volume compartment in this disease.[1] It has been shown that this regimen is often associated with a dramatic improvement in the patient's clinical condition, with rapid amelioration of the reduced cardiac output, marginal urine output, and elevated peripheral vascular resistance.[2-4] However, subjecting a patient to this treatment requires admission to an intensive care unit and hemodynamic monitoring with a Swan-Ganz catheter.[5] In addition, it is still unknown how the uteroplacental blood flow responds to relatively rapid volume expansion. Data have been reported that suggest a variable decrease[6] and increase in uteroplacental blood flow[7, 8] after volume expansion, but the employed techniques, isotope dilution[6, 7] and Doppler velocity measurements in a branch of the uterine artery,[8] provide only indirect information on uteroplacental blood flow.

From the Department of Obstetrics and Gynecology, University of Limburg.
Received for publication July 23, 1990; revised October 3, 1990; accepted October 25, 1990.
Reprint requests: Peter Focco Boekkooi, MD, Department of Obstetrics and Gynecology, Academic Hospital Maastricht, P.O. Box 1918, Maastricht, The Netherlands 6201 BX.
6/1/26355

The input for volume regulation is primarily produced in venous and atrial stretch receptors.[9] These veins also have the intrinsic capacity to dilate slowly in response to a rapid increase in intraluminal pressure, the so-called stress-relaxation phenomenon.[10] However, little is known about the impact on these two important aspects of long-term factors such as chronic venous underfilling (preeclampsia), or the chronic exposure of the veins to a certain endocrine environment (pregnancy). In previous studies on volume regulation in the awake, normovolemic rat in late pregnancy, the hemodynamic effect of gradual volume load was found to consist of a marked dilatation of large veins, an increase in cardiac output in excess of nutritional requirements, and a consistent fall in uteroplacental blood flow.[11] The reduced venous filling state and increased venous tone[9, 12] in steady-state hypovolemia are likely to cause a different hemodynamic response to a given gradual volume expansion. Particularly, the effect on the uteroplacental blood flow is uncertain. If the response is similar to that in normovolemia, uteroplacental blood flow can be expected to fall in response to volume load. On the other hand, "correction" of the hypovolemia may also reduce sympathetic tone, and thus permit a rise in uteroplacental blood flow.

The present study was designed to test the hypothesis that volume expansion in stable, hypovolemic rats in late pregnancy not only increases cardiac output and diuresis, but also uteroplacental blood flow as a result of neutralization of the hypovolemic stress. To this end, rats in late pregnancy that were mildly hypovolemic

Table I. General characteristics and baseline values (means ± SD) of the experimental animals

	Pregnant group	*Control group*	*Nonpregnant group*
Body weight (gm)	264 ± 39	252 ± 17	232 ± 22*
Urine output (ml in preceding 24 hr)	4 ± 2	18 ± 7*	10 ± 3*
Cardiac output (ml/min)	92 ± 15	109 ± 19	84 ± 21
MAP (mm Hg)	95 ± 21	89 ± 18	104 ± 12
Systemic vascular resistance (mm Hg/ml/min)	1.0 ± 0.2	0.9 ± 0.3	1.3 ± 0.4
Heart rate (beats/min)	404 ± 27	450 ± 22*	421 ± 21
Stroke volume (μl)	229 ± 43	241 ± 35	208 ± 45

*$p < 0.05$, Nonpregnant or control rats different from pregnant rats (Mann-Whitney U test).

Table II. Percentage change in hemodynamic parameters after 2 hours' hypervolemia

	Pregnant group	*Nonpregnant group*
Blood volume	+32	+35
Cardiac output	+73	+46*
MAP	NS	NS
Systemic vascular resistance	−39	−23*
Heart rate	NS	NS
Stroke volume	+68	+58

*$p < 0.05$ (Mann-Whitney U test).

because of 24 hours' thirsting, were subjected to slow, subacute volume expansion by a steady-state infusion with a plasma substitute (Haemaccel, Hoechst, Amsterdam). Cardiac output and regional blood flows were measured with microspheres before and after 2 hours' volume load. To determine the contribution of pregnancy to the volume response, the same experiments were also performed in a nonpregnant control group. The rat was considered a suitable model for this study because of its hemotrichorial placenta and its gestational rise in blood volume.[13] In addition, the hormonal changes in rat pregnancy resemble those in human pregnancy.

Material and methods

The study was performed in 20-days pregnant hypovolemic Wistar rats ($n = 7$) and nonpregnant hypovolemic Wistar rats ($n = 6$) obtained from a local breeder (HSD, Zeist, The Netherlands). A separate group of 20-days pregnant rats served as controls for the pregnant rats ($n = 7$). Three days before the experiment, the rats were anesthetized with ether, and polyethylene catheters (PE-10, outer dimension/inner dimension = 0.61/0.28, heat-sealed to a piece of PE-50 tubing, outer dimension/inner dimension = 0.92/0.58) were inserted into the right carotid artery, a femoral artery, and a femoral vein, and advanced into the left ventricle of the heart, the abdominal aorta, and the inferior vena cava, respectively. The catheters were tunneled subcutaneously and exteriorized between the shoulder blades. One day before the experiment, the rats were habituated to the large metabolism cages in which the experiments were to be performed.

After a recovery period of 2 days (in pregnant rats, day 19 of pregnancy), the rats in the pregnant and nonpregnant groups were deprived of water. After 24 hours' thirsting, the catheters were connected and elongated. Mean arterial pressure (MAP) and heart rate were monitored continuously for 30 minutes before the bolus injection until after the second injection of microspheres. Urine output was measured every 30 minutes. When heart rate and MAP had been stable for at least 30 minutes, cardiac output and its distribution were determined with 15 μm microspheres with the use of the reference sampling technique.[14] To this end, approximately 1.2×10^5 radioactive microspheres (NEN, Dreieich, Federal Republic of Germany) suspended in 0.2 ml saline solution with a drop of polysorbate 80 (0.05%) added to prevent aggregation, were injected into the left ventricle of the heart over a period of 15 seconds while a reference sample was withdrawn from the abdominal aorta at a rate of 0.5 ml/min. Reference sampling encompassed the period between 10 seconds before the microspheres injection and 30 seconds after its completion. The blood withdrawn was replaced with fresh heparinized blood from a donor Wistar rat. After the baseline microspheres experiment, an intravenous bolus injection of plasma substitute (2 ml) was administered, followed by a 2-hour infusion at a rate of 6 ml/hour. Haemaccel plasma substitute is an isotonic colloid solution composed of a polymer of urea and polypeptides. It is readily excreted in the urine with little metabolic breakdown. The agent is isotonic and does not leak appreciably into the interstitial space after intravascular administration.[15] Haemaccel plasma substitute is known to be well tolerated in the rat, with no measurable effect on MAP, heart rate, and respiratory rate, even when infused in large amounts.[16]

After 2 hours of continuous infusion, the microspheres experiment was repeated with a different radioactive label. In the control group only one blood flow measurement was performed after a stabilization period of at least 30 minutes. During the experiment changes in blood volume were calculated from the ob-

Table III. Lung fraction (percent) and regional blood flows (milliliters per minute per 100 gm) before and after 2 hours' hypervolemia

	Pregnant group		*Control group baseline*	*Nonpregnant group*	
	Baseline	*2 hrs*		*Baseline*	*2 hrs*
Lung fraction	3.7 ± 1.4	1.7 ± 0.6*	1.7 ± 1.6	1.9 ± 0.7	2.2 ± 1.0
Organ flows					
Kidneys	10 ± 2	23 ± 5*	12 ± 4	11 ± 3	20 ± 6*
Intestines	20 ± 4	28 ± 8	22 ± 8	14 ± 4	20 ± 6*
Portal	21 ± 4	33 ± 7*	24 ± 8	15 ± 5	24 ± 8*
Brain	2.6 ± 0.7	3.5 ± 0.7	3.0 ± 1.0	3.0 ± 1.0	4.1 ± 1.7
Skin	9 ± 2	10 ± 4	11 ± 3	7 ± 1	8 ± 3
Carcass	27 ± 7	39 ± 10*	32 ± 8	32 ± 17	36 ± 15
Myometrium	2.1 ± 0.7	2.0 ± 0.6	1.9 ± 0.8	0.6 ± 0.3†	0.4 ± 0.2
Placentas	2.9 ± 1.3	2.1 ± 1.5	4.4 ± 1.1	—	—

*$p < 0.05$, Wilcoxon signed rank test.
†$p < 0.05$, Mann-Whitney U test.

served changes in hematocrit (hematocrit, volume percentage, microcapillary technique). In a preceding validation study in five nonpregnant rats, plasma volume and red cell volume were determined before and after volume expansion on the basis of the indicator (albumin, labeled with iodine 125, and erythrocytes, labeled with chromium 51, respectively) dilution technique.[17] Blood volume changes calculated from the isotopes data and those based on the simultaneously measured change in hematocrit, differed by less than 5%. This result together with the minimum need for blood sampling and no requirement for the use of isotopes, led us to adopt the hematocrit method for the estimation of blood volume changes in the present study.

After the second microspheres experiment, the animals were killed with an overdose of pentobarbital. All organs were dissected and weighed. The radioactivity in each tissue aliquot and reference sample was determined with a sodium crystal scintillation counter (Packard, Groningen). Cardiac output was calculated as follows:

$$\text{Cardiac output (milliliters/minute)} = \frac{\text{Counts per minute in total body} \times \text{Reference sampling rate}}{\text{Counts per minute in reference sample}}$$

Regional blood flows were then calculated as cardiac output × fractional entrapment of microspheres as described in detail elsewhere.[18] Mixing of the microspheres after injection was considered adequate in all animals on the basis of a less than 5% difference in fractional entrapment of microspheres in the two kidneys.[19] The number of microspheres recovered in the reference samples ranged from 400 to 700.

Data are presented as means ± SD. Paired data obtained after volume load and in baseline were evaluated with the Wilcoxon signed rank test. Differences between pregnant and nonpregnant rats and between pregnant and control rats were tested by the Mann-Whitney U test. A probability of <5% (two-sided) was considered significant.

Results

One day of water deprivation decreased blood volume by 7% in both groups. Table I lists the baseline values for cardiac output, MAP, systemic vascular resistance, stroke volume, and body weight after 1 day of hypovolemia and those in the control group. Pregnant rats were heavier than their age-matched nonpregnant countermates. Neither by paired analysis nor by comparison of data of the pregnant rats with the control group did any of the hemodynamic variables change in response to the water deprivation. The results of this analysis were comparable to pregnant and nonpregnant rats.

Volume expansion increased blood volume by 32% and 35% in the pregnant group and the nonpregnant group, respectively (Table II). In the pregnant and nonpregnant groups, volume load increased cardiac output by 73% and 46%, and decreased systemic vascular resistance by 39% and 23%, respectively. The change in these two variables was smaller in the nonpregnant group than in the pregnant group. The rise in cardiac output was mainly a result of a rise in stroke volume. Neither MAP nor heart rate changed appreciably in the pregnant and nonpregnant groups.

The lung fraction and organ flows before and after volume expansion and those in the control group are listed in Table III. The volume load induced a rise in renal flow by 127% and 94% in the pregnant group and nonpregnant group, respectively. Portal flow increased by 60% in both groups, whereas carcass flow increased only in the pregnant group. Blood flows to brain, skin, and myometrium did not change consistently in either group. Fig. 1 summarizes the results and shows the distribution of the extra cardiac output

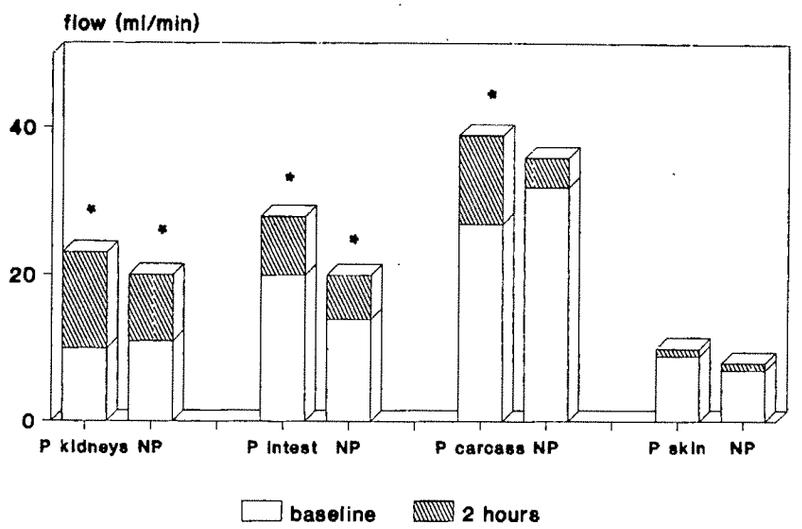


Fig. 1. Distribution of extra cardiac output after 2 hours' hypovolemia. In each pair of bars, *left bar* represents pregnant *(P)* group, and *right bar* represents nonpregnant *(NP)* group. *Asterisk* indicates significant change from baseline.

Table IV. Percentage change in hemodynamic variables in response to volume load in normovolemic and hypovolemic pregnant rats

	Pregnant state		*Nonpregnant state*	
	Normovolemic	*Hypovolemic*	*Normovolemic*	*Hypovolemic*
Fluid retention (ml)	9.3	11.7*	9.1	10.2
Blood volume	+19	+32*	+22	+35
Cardiac output	+43	+73*	+45	+46
MAP	NS	NS	NS	NS
Heart rate	NS	NS	NS	NS
Systemic vascular resistance	−35	−39	−33	−23
Organ flows				
Kidneys	+76	+127*	+87	+94
Portal bed	NS	+64*	+37	+46
Carcass	+81	+48	+28	NS
Placentas	−40	−31	—	—

*Percentage change in hypovolemia different from that in normovolemia (Mann-Whitney U test).

in both groups. The consistent fall in fung fraction (≈ 2%) after the volume load only in the pregnant group, may at least in part be caused by secondary dislodgment of microspheres from the placental microcirculation. This phenomenon causes discrete but inconsistent underestimation of the baseline uteroplacental blood flow. Therefore uteroplacental blood flow after 2 hours infusion in the pregnant group was compared with uteroplacental blood flow in the control group. Lung fraction, litter size, fetal weight, and placental weight were comparable in the pregnant and the control groups. Uteroplacental blood flow markedly decreased in the pregnant group as compared with the value observed in the control group (180 ± 26 and 80 ± 22 ml/min per kilogram fetus, respectively, $p < 0.001$).

Finally, the magnitude of the changes induced by the volume load in the present study was compared with that previously observed in normovolemic pregnant ($n = 7$) and nonpregnant rats ($n = 6$) subjected to the same gradual volume load (Table IV). The hypovolemic pregnant rats showed a stronger hemodynamic response than that of their normovolemic countermates. This difference was not observed in the nonpregnant rats.

Comment

Maternal hypovolemia induced by 1-day water deprivation leads to gradual dehydration that includes both depletion of the extravascular space and a 7% reduction in blood volume in both pregnant and nonpregnant rats. The impact of this reduction on he-

modynamics is likely to be larger in the pregnant group inasmuch as the physiologic blood volume expansion is reversed to blood volume reduction. The constricted inferior vena cava, which was shown on x-ray film in our laboratory, complies with a decreased venous capacity after 24 hours' thirsting. It probably indicates compensatory mobilization of unstressed blood volume from the venous capacitance vessels to maintain venous return.[20] The lack of a change in cardiac output and organ flows in hypovolemia illustrates the efficacy of this compensation.

Table IV illustrates that gradual volume expansion in the mildly hypovolemic pregnant rat triggers a similar pattern of changes in cardiac output, MAP, systemic vascular resistance, heart rate, and organ flows in the normovolemic state. However, there were a number of distinct quantitative differences. First, gradual volume expansion led to a larger fluid retention in the hypovolemic rat than that in the normovolemic pregnant rat. Second, only in pregnant rat the induced cardiac output response in the hypovolemic state was significantly larger than that in the normovolemic state. This indicates that the volume load in the hypovolemic pregnant rat triggers a larger rise in stressed volume than that in the normovolemic rat. The slow onset of diuresis (after ≈ 60 instead of 20 minutes) indicates a delayed diuretic response, giving rise to a larger volume retention. This is reflected in a larger blood volume expansion in the hypovolemic (32%) than in normovolemic state (19%). Expansion of unstressed volume is likely to be limited by the elasticity of the veins, irrespective of their initial filling state.[9, 10, 21] It follows that a larger blood volume expansion can be expected to affect primarily the stressed volume compartment. In the pregnant rats, 24 hours' thirsting induced a stronger antidiuretic response than that in the nonpregnant rats (Table I). This may explain the longer delay in the onset of diuresis and larger volume expansion in response to volume expansion in the pregnant rats.

Uteroplacental blood flow also decreased consistently in the hypovolemic pregnant animal, similar to what was previously seen in the normovolemic rat. The mechanism responsible for this response is obscure. By microscopic inspection of the placentas of three pregnant rats that had been subjected to this particular volume load and the placentas of three control pregnant rats, it was shown that volume loading caused consistent selective placental congestion without concomitant distension of the venous plexus. One can speculate that gradual volume expansion and associated central venous dilatation may be paralleled by an increase in the venous resistance to flow, a phenomenon also described for preeclamptic pregnancy.[12] The rat placenta lacks venules and is equipped with rectangularly branching microchannels that cannot be opened or closed selectively. The perfusion of such a peculiar microvasculature is likely to be extra vulnerable to a rise in venous resistance. Besides, a further reduction in the already low shear forces can be expected to undermine local blood rheology, causing red blood cell sludging and enhanced formation of red blood cell aggregates.[22]

These results can be used to speculate about the hemodynamic effects of volume expansion therapy in the preeclamptic patient. In doing so, one should keep in mind that the present model differs in important aspects from the preeclamptic hemodynamic condition. The hypovolemic state in these rats is paralleled by a volume-depleted interstitium, normal blood pressure, normally grown fetuses and placentas, and normal volume regulation. The microcirculation is not impaired by enhanced capillary leakage. On the other hand, preeclampsia often couples increased venous tone and reduced venous capacitance[12] with impaired renal function. This combination increases the risk for overcorrection, defined as volume expansion in excess of maximal venous capacity. This overcorrection seems to be particularly unfavorable for uteroplacental blood flow. It follows that magnitude of diuretic response and increase in cardiac output provide incomplete information about whether volume expansion has led to correction or overcorrection of the hypovolemic state. Whether this overcorrection can be prevented by reduction of the infusion rate needs further study.

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Pharmacokinetics of pentamidine in Sprague-Dawley rats in late pregnancy

Bertis B. Little, MA, PhD, Timothy H. Harstad, MD, Roger E. Bawdon, PhD, Sohrab Sobhi, BS, Daniel A. Roe, BS, Kraig A. Knoll, BS, and Fred E. Ghali, BS

Dallas, Texas

To our knowledge, placental transfer of pentamidine has not been previously studied in vivo. In the present study, the pharmacokinetics of pentamidine were analyzed in late gestation (18 days) among Sprague-Dawley rats. Pentamidine's kinetics were assessed in the following maternal compartments over a 12-hour period in 16 timed-pregnant rats: serum, liver, and kidney. Placentas were also analyzed for pentamidine concentration as were fetal brain, liver, and kidney tissues. Significant placental transfer of the drug was found, with pentamidine reaching all fetal compartments studied. Notably, by the twelfth hour fetal brain tissue achieved pentamidine concentrations that were not significantly different from those of maternal serum at the second hour of the experiment. This is an interesting observation because adult mouse and rat brains were found to be unexposed to the drug. (AM J OBSTET GYNECOL 1991;164:927-30.)

Key words: Pentamidine, pharmacokinetics, pregnancy, Sprague-Dawley rats

Pentamidine isethionate is used to treat *Pneumocystis carinii* infections in both pregnant and nonpregnant patients. Placental transfer of the drug was investigated in vitro with the perfused human placenta model. In that study,[1] therapeutic concentration of the drug was found in the placental tissue, although little drug was found in the fetal perfusate. Whether this in vitro model adequately reflects kinetics of pentamidine in vivo is unclear.

The purpose of the present study was to analyze the pharmacokinetics of pentamidine in the maternal compartment, placenta, and fetal compartment in an in vivo animal model.

Material and methods

This study protocol was reviewed and approved by the Institutional Review Board for Animal Research at the University of Texas Southwestern. The rat was chosen as the animal model because this animal showed similarity in placental transfer of another antibiotic, azidothymidine, to human beings,[2] the size is convenient for handling and dissection, and the rats have a known heritage for many generations and are genetically homogeneous. Sixteen timed-pregnant Sprague-Dawley rats were obtained from a commercial vendor (Sasco Inc., Houston) on day 9 to 10 of pregnancy, and maintained on water and Purina Rat Chow as desired until day 18 of pregnancy. On day 18 of pregnancy, rats were injected with 40 mg/kg of pentamidine in the tail vein. Four rats each were assigned to four treatment groups and were killed by decapitation at 2, 6, 8, or 12 hours after the tail vein injection with the drug. After the rats were killed, maternal samples (serum, liver, kidney), placenta, and fetal tissues (brain, liver, kidney) were collected. Fetal samples from the same mother were combined to obtain enough tissue for analysis of drug concentration. Hence, fetal measurements represent litter values and not those of individual fetuses. It was not possible to collect fetal serum samples because of the small size of rat fetuses, which is a limitation of the animal model.

The tissue was rinsed in 0.1 mol/L phosphate buffer, blotted, and approximately 0.5 gm of tissue was weighed and 2 volumes of 0.1 mol/L phosphate buffer with a pH of 6.1 was added. The samples were then homogenized with a Tissuemizer (Tekmar Co., Cincinnati), centrifuged, and injected directly into the high-performance liquid chromatography with no extraction. The methods of procedure for high-performance liquid chromatography determination of pentamidine used were as described previously without modification.[1] Standards were prepared with standard assay pentamidine isethionate provided by Lyphomed Corporation, Chicago. A standard curve was prepared in 0.1 mol/L phosphate buffer with a pH of 6.1 and these data were calculated with linear least-squares regression analysis ($R^2 = 0.98$).

Newman-Keuls multiple comparison test for analyzing the significance of differences between several means was used to analyze the pharmacokinetics data.

From the Division of Prenatal Diagnosis, Department of Obstetrics and Gynecology, The University of Texas Southwestern Medical Center.
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Reprint requests: Bert Little, MD, Division of Prenatal Diagnosis, Department of Obstetrics and Gynecology, The University of Texas Southwestern Medical Center, Dallas, TX 75235-9032.
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Table I. Descriptive statistics for pentamidine concentrations (μg/ml) in maternal and fetal tissues and the placenta

Time (min)	*Fetal brain*			*Fetal kidney*			*Fetal liver*		
	N	*Mean*	*SD*	*N*	*Mean*	*SD*	*N*	*Mean*	*SD*
120	4	11.6	5.9	4	11.7	1.5	4	11.5	2.5
360	4	3.0	1.5	4	14.3	2.1	4	8.3	3.0
480	4	10.3	3.7	4	16.8	1.8	2	3.6	3.2
720	2	13.3	0.5	3	10.3	2.2	4	4.9	1.7

N, Sample size.

Table II. Newman-Keuls multiple comparison analysis of differences in concentration (μg/ml) within time periods between tissue

	Maternal serum								*Placenta*					
	vs Fetal brain		*vs Fetal kidney*		*vs Fetal liver*		*vs Placenta*		*vs Fetal brain*		*vs Fetal kidney*		*vs Fetal liver*	
Time (min)	*Q*	*p*	*Q*	*p*	*Q*	*p*	*Q*	*p*	*Q*	*p*	*Q*	*p*	*Q*	*p*
120	6.5	0.05	6.5	0.05	6.6	0.05	8.8	0.05	2.9	NS	2.3	NS	2.3	NS
360	2.5	NS	0.6	NS	1.1	NS	1.5	NS	0.8	NS	1.7	NS	0.4	NS
480	0.8	NS	3.1	0.05	4.8	0.05	6.1	0.05	4.4	0.05	7.6	0.05	0.8	NS
720	3.2	0.05	4.5	0.05	6.7	0.05	6.6	0.05	4.4	0.05	2.1	NS	0.1	NS

Q, Newman-Keuls Q statistic; *p*, significance.

Testing was performed with UTSTAT computer software on the VAX 8800 computer at the University of Texas Southwestern Medical Center. Unpaired analysis was used.

Results

Means and SDs of the concentrations of pentamidine in various maternal and fetal tissues are given in Table I. Newman-Keuls multiple comparison test of differences between tissues within a specific time period are given in Table II. Two hours after injection, maternal serum concentrations of pentamidine were significantly higher than those in the placenta or any fetal tissue Table II). During the same time period, maternal kidney concentration of the drug was significantly higher than that of the placenta, but was not different from that of the fetal kidney. Maternal serum concentration of pentamidine was significantly higher than that of the maternal kidney. Maternal liver concentration of the drug was not different from that of the maternal serum, but was significantly higher than that of the fetal liver or the placenta. Six hours after administration of pentamidine, no tissue concentrations, fetal or maternal, were statistically different from maternal serum concentrations of the drug. Eight hours after intravenous injection of pentamidine, fetal brain, maternal kidney, and liver maintained pentamidine levels not significantly different from that of maternal serum. Fetal kidney, liver, and the placental concentrations of pentamidine were significantly lower than that of maternal serum. The concentration of pentamidine in maternal kidney was not significantly different from that of maternal serum, but this may be attributed to the large SD of kidney in this time period (Table I).

Fetal brain concentrations of the drug were increasing whereas in other tissues concentrations were decreasing (Table II), although the levels oscillated, probably reflecting the fact that pentamidine is biphasic. By 6 hours after injection, fetal brain achieved pentamidine levels not significantly different from those of maternal serum, and maintained high levels until the conclusion of the experiment. Fetal kidney concentrations of pentamidine suggest the fetus may be excreting large amounts of the drug because levels in the organ slightly exceeded those in the maternal serum and maternal kidney at 6 to 8 hours after injection, consistent with a biphasic pharmacodynamic effect. Fetal liver pentamidine concentrations paralleled those of the placenta, whereas drug concentrations in maternal liver were similar to those in maternal serum. It is of interest that placental pentamidine concentrations were less than those of all fetal or maternal tissue levels. Consistent with reports of the drug's half-life, concentrations of pentamidine in maternal serum decreased by about one half in the first 6 hours. Concentrations in maternal serum increased in the period 8 to 12 hours, perhaps because of fetal-to-maternal transfer of the drug, as indicated by the rise in concentrations of pentamidine in fetal kidney at 6 to 8 hours and in the placenta from 8 to 12 hours. However, the biphasic nature of pentamidine may also explain such pharmacodynamic behavior of the drug. Consequently at 12 hours after

Placenta			*Maternal kidney*			*Maternal liver*			*Maternal serum*		
N	*Mean*	*SD*	*N*	*Mean*	*SD*	*N*	*Mean*	*SD*	*N*	*Mean*	*SD*
4	4.0	1.9	3	12.4	1.2	3	34.8	11.1	2	33.3	2.9
2	6.6	0.2	2	10.7	5.5	4	12.0	5.1	4	12.2	3.5
4	1.5	0.7	4	11.0	5.5	4	9.4	4.4	4	11.6	0.5
4	5.2	2.2	2	8.7	8.3	3	8.7	2.4	4	21.1	4.0

Maternal kidney						*Maternal liver*					
vs Fetal kidney		*vs Maternal serum*		*vs Placenta*		*vs Fetal liver*		*vs Maternal serum*		*vs Placenta*	
Q	*p*	*Q*	*p*	*Q*	*p*	*Q*	*p*	*Q*	*p*	*Q*	*p*
0.4	NS	10.5	0.05	4.2	0.05	3.9	0.05	0.3	NS	5.2	0.05
1.1	NS	0.4	NS	1.2	NS	1.1	NS	0.1	NS	1.6	NS
2.0	NS	0.2	NS	3.2	0.05	2.1	NS	0.8	NS	2.9	NS
0.3	NS	2.6	NS	0.7	NS	1.4	NS	4.6	0.05	1.3	NS

injection, all fetal tissues and the placenta were significantly lower than maternal serum.

Maternal brain concentrations of pentamidine were measured and we concluded there was no significant penetration of the blood-brain barrier. The minimum detectable level of the drug in tissue was 0.5 μg/ml. The concentration of pentamidine in maternal brain was 1.1 ± 1.3 (mean ± SEM) μg/ml, which is 3% of the maternal serum level. Because these levels are very close to the minimum sensitivity of the high-performance liquid chromatography method, it is possible that maternal brain tissue levels of pentamidine were interfering substances (i.e., blood contamination of the brain tissue samples). We conclude that maternal brain achieved drug concentrations of pentamidine not significantly different from zero.

Comment

Pentamidine readily crossed the rat placenta and reached concentrations as high as maternal serum levels in several key fetal tissues (i.e., brain and kidney), but this may be a function of the relatively large doses used in the study (i.e., 10 times the therapeutic human intravenous dose). It is possible this high-dose regimen was able to override the protective effect of the placenta that was hypothesized in an earlier in vitro study.[1] Whereas the placenta allowed pentamidine to cross, concentrations of the drug in this tissue were invariably lower than those of other organs, maternal or fetal. Generally, drug concentrations decreased over time, with one exception, the fetal brain, which experienced a biphasic increase over time. This indicates that pentamidine readily penetrates the fetal blood-brain barrier, which is sharply different from the adult mouse brain in which no pentamidine was found over time,[5] and our results that suggested no significant penetration of the adult rat blood-brain barrier. The implications of this finding for embryo-fetal development are not known.

The high concentrations of pentamidine in the fetus may be of clinical interest. In a previous study that used pregnant animals given pentamidine over an extended period of time, investigators found a significantly increased frequency of embryonic resorptions, but no congenital anomalies.[4] It is possible that these embryonic deaths were a result of early damage of vital systems by persistent levels of pentamidine. In the human being, pentamidine is now given in an aerosolized form by inhalation, resullting in much lower serum concentrations than when the drug is administered by other routes. Pharmacokinetics of the drug at these lower doses have not been studied, but may follow a similar pattern.

Caution must be used when extrapolating findings of animal studies of both placental transfer and teratogenicity to conditions in human beings because the composition of the rat placenta and development of the rat conceptus are different from human placenta and conceptus. Human and rat placentas are both hemochorial, but in the rat there is a layer of cellular trophoblast interposed between the chorionic villus and maternal blood. This may cause species differences in

the drug's kinetics across the placenta. Despite species differences in placental morphology, the results of this study are similar to findings in in vitro perfused human placental lobules.[1]

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Thumbtacks

To the Editors: I am writing to support the use of thumbtacks to obtain hemostasis in cases of intractable venous sacral hemorrhage as advocated by Patsner and Orr (Patsner B, Orr JW Jr. Intractable venous sacral hemorrhage: use of stainless steel thumbtacks to obtain hemostasis. AM J OBSTET GYNECOL 1990;162:452).

We obtained radiographs after surgery in a patient whom we treated in November 1987 (Figs. 1 and 2). At that time, she was a 42-year-old gravida 14, para 13-0-12, white woman at 35 weeks' gestation with a 2 cm stage IB (International Federation of Gynecology and Obstetrics) squamous carcinoma of the cervix. A low vertical cesarean section and radical hysterectomy with bilateral salpingo-oophorectomy and pelvic and paraaortic lymphadenectomy were performed. When the retroperitoneal spaces were opened, massive pelvic venous varicosities were encountered. These vessels were fastidiously avoided, but retractor trauma resulted in presacral bleeding that could not be controlled with pressure or suture ligation. Three thumbtacks were sterilized and carefully placed in the presacral area with

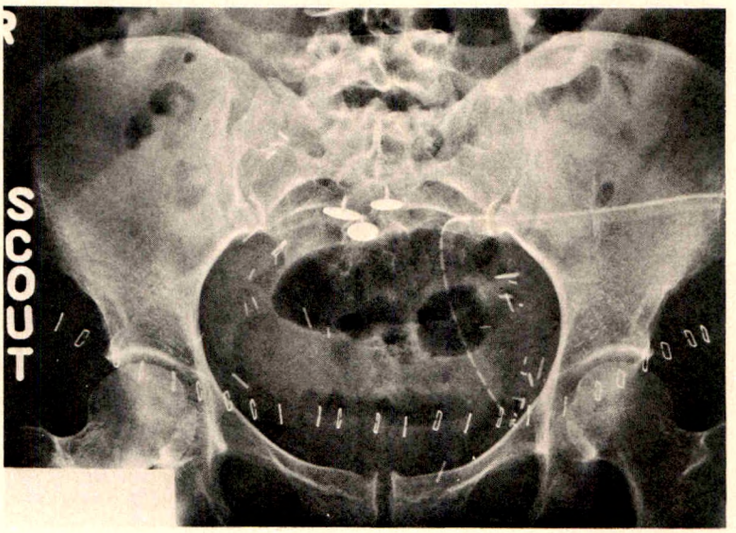


Fig. 1. Kidney and upper bladder radiograph shows thumbtacks in anterior sacrum.

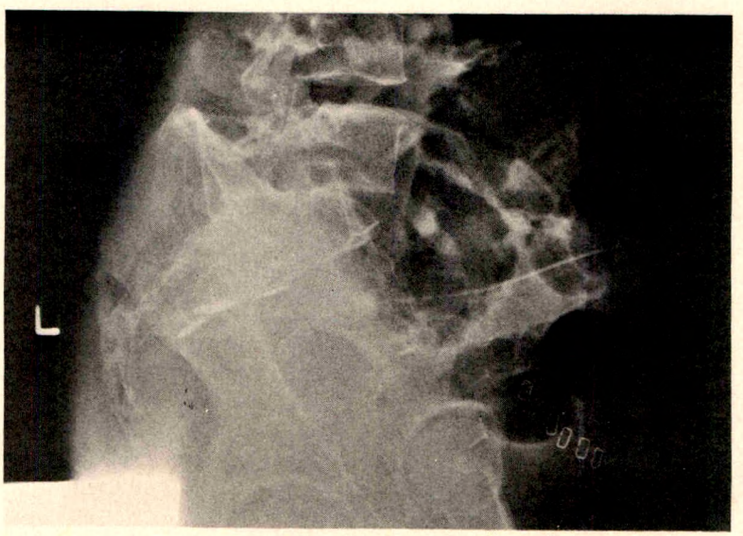


Fig. 2. Lateral radiograph shows thumbtacks in anterior sacrum.

pressure from a Kelly clamp. Hemostasis was achieved rapidly. This patient recovered uneventfully despite an operative blood loss of 10,000 ml. She has been free of cancer for 3 years and has no symptoms that can be attributed to the thumbtacks.

As a surgeon who is familiar with such cases,[1] I heartily endorse the timely use of sterile thumbtacks in the case of massive presacral bleeding.

Gregory P. Sutton, MD

Department of Obstetrics and Gynecology, Indiana University Hospital, 926 W. Michigan St., Indianapolis, IN 46202

REFERENCE

1. Sutton GP, Addison WA, Livengood CH, Hammond CB. Life-threatening hemorrhage complicating sacral colpopexy. Am J Obstet Gynecol 1981;140:836-7.

The importance of the postcoital test

To the Editors: I read with interest the article by Griffith and Grimes (Griffith CS, Grimes DA. The validity of the postcoital test. Am J Obstet Gynecol 1990; 162:615-20.) on the validity of the postcoital test. They point out to the reader the problems in interpretation of previous articles in the literature: variation existed in standardization for the interval after intercourse, definition of an abnormal postcoital test, timing in the cycle of the postcoital test, and degree of investigation of other factors that could lead to infertility.

The emphasis of these shortcomings should certainly make the reader at least question some of the previous dogmatic statements that were made about the clinical importance of the postcoital test and should encourage clinicians and researchers to perform prospective randomized investigations to determine whether performance of this test is a waste of time or money.

However, instead of merely highlighting the caveats, the authors proceeded to use the same discordant studies to leave the reader with the impression that the postcoital test has poor validity as a diagnostic test for infertility and therefore to encourage the clinician to abandon the test, rather than to refine the postcoital examination and then assess its clinical importance. They suggest, in fact, that at least $50,000,000 is wasted each year on a test with poor validity.

We previously presented data concerning the importance of the use of follicular maturation studies to be certain of proper timing of the examination and to be sure that poor quality mucus is not related to failure to form a mature follicle (inadequate estrogen effect) or premature luteinization (adverse progesterone effect).[1] We, like Hull et al.,[2] define abnormal as the absence of progressively moving sperm in the mucus 8 to 12 hours after intercourse. In one study of 70 patients whose only apparent infertility problem was a cervical factor, only 2 of 20 patients (10%) conceived when the test was not corrected compared with 37 of 50 patients (74%) in whom at least some sperm with progressive forward motion were noted in the mucus.[3]

One problem with the conclusions from the ethinyl-estradiol-human menopausal gonadotropin study was that even though mature follicles were demonstrated at the time of the postcoital tests, the possibility existed that hyperstimulation might have been responsible for the pregnancy rather than improvement of the sperm-mucus interaction. Recently, however, we presented data at the Seventh World Congress on Human Reproduction in Helsinki, Finland, which were based on nonstimulated cycles with 0 moving sperm. After intercourse there was only a 3.9% pregnancy rate per cycle compared with 21.2% with properly timed intrauterine insemination.

I write this letter to caution the reader not to abandon the postcoital test but to strive to be careful about potential pitfalls in misinterpretation and to be open-minded about the importance of the test. Until the authors or some other researchers present clear prospective data that negate the clinical importance of this test, I believe that conclusions about the invalidity of the test, which are based on other studies that lack standardization, are in themselves invalid.

Jerome Check, MD

The UMDNJ Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Camden, NJ 08103

REFERENCES

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Reply

To the Editors: Dr. Check seems to agree with our major points, yet he reaches a different conclusion than we did. He criticizes our having "proceeded to use these same discordant studies" for assessment of the validity of the postcoital test. Regrettably, these are the best available data. Quantitative evaluation of the validity of this test was long overdue.

We agree about the importance of good cervical mucus. As we stated in our article, we restricted our analysis to only those patients with good cervical mucus to control for this potentially distorting factor. Dr. Check's other comments about the ethinyl-estradiol-human menopausal gonadotropin study and the data that were presented in Helsinki do not relate to validity.

Dr. Check's final paragraph is puzzling. He agrees that there are "potential pitfalls," about which one must be "careful." All the studies that we analyzed were prospective in design; taken together, they suggest that the test lacks validity. He neither disputes our calculations nor offers other studies that contradict our findings. However, he believes that no conclusions can be drawn. Is this being "open-minded about the importance of the test?"

Within several years of its introduction, the enzyme-linked immunosorbent assay test for the human immunodeficiency virus was shown to be highly sensitive and specific as well as clinically useful.[1, 2] In contrast, the postcoital test has been used widely for more than 120 years, yet objective evidence of its validity, reliability, and utility is lacking. If millions of tests and a century of experience have not demonstrated its value, what is the probability that a few more decades of use will?

When we choose tests and procedures, we must be sure that they are worth the expense, pain, and inconvenience that they cause. In a discussion of protection of the "medical commons" from "overgrazing" by tests and procedures of no value or of undetermined value, the Dean of the Harvard School of Public Health[3] cited many examples of practices that were once widely used but have now disappeared. Because they remained on the "medical commons" too long, valuable health resources were squandered. Widespread postcoital testing draws money and personnel away from other areas of medicine. If these other areas of medicine are more important, then the testing has a negative effect on public health.

David A. Grimes, MD, and Carolyn S. Griffith, MD
Department of Obstetrics and Gynecology, University of Southern California School of Medicine, Women's Hospital, 1240 N. Mission Road, Los Angeles, CA 90033

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First-trimester transvaginal ultrasonographic screening: Incidence of structural anomalies

To the Editors: I read with interest the article "Transvaginal ultrasonographic diagnosis in the first trimester" by Cullen et al. (Cullen MT, Green J, Whetham J, Salafia C, Gabrielli S, Hobbins J. AM J OBSTET GYNECOL 1990;163:466-76). The 33 embryos identified as having structural anomalies in the 622 patients studied makes the incidence of structural anomalies 5.3%, not 0.5% as the authors state. This percentage now becomes unusually high rather than the "surprisingly low" incidence they cite in their discussion. How would the argument change in the light of the correct statistics? What could be the reason for scanning a low-risk population and obtaining such a remarkably high incidence of anomalies?

The last sentence of the article, in which the authors state, "Prenatal diagnosis of structural anomalies will move into the first trimester," may be a premature consideration.[1] Structural anomalies revolving around secretion, passage, or absorption of systemic fluids (e.g., cerebrospinal fluid, urine, or swallowed amniotic fluid) or others such as microcephalus, agenesis of corpus callosum, or cleft palate may not be detected during the first trimester, and the list is long.

Therefore I would not move ultrasonographic diagnosis of structural anomalies into the first trimester with the knowledge that many anomalies will develop during the second trimester.

Shraga Rottem, MD, DSc
Department of Obstetrics and Gynecology, Rambam Medical Center, 31096 Haifa, Israel

REFERENCE

1. Rottem S, Bronshtein M. Transvaginal sonographic diagnosis of congenital anomalies between 9 weeks and 16 weeks, menstrual age. JCU 1990;18:307-14.

Reply

To the Editors: Dr. Rottem has correctly pointed out an error in decimal placement. Twenty-two of the 33 fetal anomalies (66%) occurred in patients with a high risk of congenital anomaly. The remaining 11 were from a relatively low-risk population. This incidence is lower than would be expected for the general population at term and lower than our expectations, when the higher incidence of aneuploidy in the first trimester is considered.

Dr. Rottem is concerned that by moving prenatal diagnosis into the first trimester some anomalies may be missed. We emphasized in the manuscript the possible limitations of early ultrasonographic diagnosis and cited specific examples. However, this should not stop investigators from attempting early diagnosis. We certainly do not advocate a single first-trimester transvaginal examination as a final screening test.

Mark T. Cullen, MD
Director, Prenatal Diagnosis Program, Department of Obstetrics and Gynecology, University of Florida, Jacksonville, FL 32209

Metabolic effects of danazol

To the Editors: I was interested to read the report entitled "Insulin-dependent diabetes mellitus associated with danazol" (Seifer DB, Freedman LN, Cavender JR, Baber RA. AM J OBSTET GYNECOL 1989;162:474-5.) Two points are worthy of mention.

First, the initial appearance of symptomatic hyperglycemia seems very likely to have been associated with danazol treatment, but the criteria for defining the patient's diabetes as "insulin-dependent" are not clear. The weight loss of 2 pounds is trivial and can be explained by day-to-day variation, and there is no mention of whether the patient had significant ketosis. Also, I note that she tested negative for islet cell antibodies. Moreover, true insulin-dependent diabetes can enter a "honeymoon phase" of improved glycemic control and reduced insulin requirements, but it would be very unusual for a patient to be able to stop taking insulin completely for several months, as in this case. The most

likely diagnosis in this patient is, in fact, *non*-insulin-dependent diabetes, which was presumably precipitated by danazol-induced insulin resistance and which was treated with insulin. It is probable that the patient would have responded to caloric restriction alone, which would not have been provided by the 2200-calorie diet that is designed for insulin-dependent diabetics.

The second point concerns the cause of danazol-induced hyperglycemia. Danazol causes postreceptor resistance to insulin, which is attributed to the androgenic properties of the drug.[1] Another possible action is through stimulation of glucagon secretion: danazol can induce hyperglucagonemia of the degree that is usually associated with glucagonoma.[2] This would both aggravate insulin resistance and stimulate insulin secretion, which, if the β-cell reserve were already compromised, might lead to exhaustion of insulin secretion and thus to non-insulin-dependent diabetes. A group of danazol-treated patients whom we described previously[2] were glucose-intolerant overall, and one patient had impaired glucose tolerance according to World Health Organization criteria. None of the patients in this group had any hyperglycemic symptoms, and the patient with impaired glucose tolerance reverted to normoglycemia after withdrawal of the drug. Stimulation of glucagon secretion may be an androgenic property, because oxymetholone can also cause "pseudoglucagonoma."[3]

In conclusion, it is clearly important to monitor glycemia in women who are being treated with danazol. Practitioners who prescribe this drug must also be aware that it can cause gross hyperglucagonemia. Recognition of this assocation may spare patients with danazol-induced "pseudoglucagonoma" from undergoing unnecessary and often invasive investigations.[4]

Gareth Williams, MA, MD
Department of Medicine, The University of Liverpool, P.O. Box 147, Liverpool, England L69 3BX

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1. Wynn V. Metabolic effect of danazol. J Int Med Res 1977;5 (suppl 3):25-35.
2. Williams G, Lofts F, Füessl H, Bloom SR. Treatment with danazol and plasma glucagon concentration. Br Med J 1985;291;1155-6.
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4. David J. Hyperglucagonaemia and treatment with danazol for systemic lupus erythematosus. Br Med J 1985;291: 1170-1.

Reply

To the Editors: Although it is possible that our patient had non-insulin-dependent diabetes mellitus, we favored a diagnosis of insulin-dependent diabetes in light of 3+ ketones in the urine, which was accompanied by a random blood glucose level of 461 mg/dl and symptoms that required insulin for control. We acknowledge the possible presence of hyperglucagonemia as a result of danazol therapy, which may have exacerbated her symptoms.

David B. Seifer, MD
Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510

Fetal subdural hematoma before labor

To the Editors: We are interested in the case report by Demir et al. (Demir RM, Gleicher N, Myers SA. Atraumatic antepartum subdural hematoma causing fetal death. Am J Obstet Gynecol 1989;160:619-20) but cannot agree with their statement that "No previous report has documented the presence of an atraumatic subdural hematoma resulting in fetal death before labor."

In 1984 we described 16 stillborn infants who had large, often bilateral, subdural hematomas.[1] The maternal histories suggested that fetal deaths occurred at a mean of 10 days before the macerated infants were delivered at gestations from 26 to 41 weeks. All mothers were immigrants from the Pacific Islands. We studied an additional 27 stillborn Pacific Islander infants with similar subdural hemorrhages that preceded the onset of labor and are still not certain of the cause.[2] There was no history of trauma or anticoagulation therapy and coagulation and platelet counts were normal in studies of the mothers and two similarly affected liveborn infants.[3]

Isoimmune thrombocytopenic purpura is one of the few coagulation disorders known to be associated with intracranial hemorrhages before delivery[4] and Demir et al. do not state whether this was excluded in their case by showing the absence of maternal antibodies specifically directed against paternal platelets. Demir et al. suggest that the hemorrhage was the cause of chronic hydrocephalus in their case in which a dilated third ventricle was consistent with obstruction of the aqueduct of Sylvius. We reported two cases of congenital hydrocephalus associated with fetal intracranial hemorrhage, and in one there was evidence that hemorrhage caused narrowing of the aqueduct.[5] Other infants showed effects of intrauterine blood loss such as edema or erythroblastosis.

We agree with Demir et al. that fetal subdural hematoma may be unrelated to birth trauma, but we are cautious about excluding other trauma on the basis of maternal history alone. There are many situations in which trauma to the mother might be denied subsequently and we speculate that the large number of cases in Pacific Islanders is caused by traditional forms of abdominal massage during pregnancy.[2] The condition is much less common in other populations in New Zealand but recently a 35-year-old European woman was delivered of an 800 gm male stillborn infant with a large subdural hematoma. We have seen liveborn infants with the consequences of similar hemorrhages and we agree that it is important for both medical and

legal reasons that birth trauma not be assumed to be the cause.

T.R. Gunn, MD

Department of Paediatrics, University of Auckland, School of Medicine, Park Road, Auckland, New Zealand

D.M.O. Becroft, MD

Department of Pathology, University of Auckland, School of Medicine, Park Road, Auckland, New Zealand

REFERENCES

1. Gunn TR, Becroft DMO. Unexplained intracranial haemorrhage in utero: the battered fetus? Aust NZ J Obstet Gynaecol 1984;24:17-22.
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5. Gunn TR, Mora JD, Becroft DMO. Congenital hydrocephalus secondary to prenatal intracranial haemorrhage. Aust NZ J Obstet Gynaecol 1988;28:197-200.

Reply

To the Editors: We appreciate the interest that Drs. Gunn and Becroft expressed in our earlier work. Whereas we were aware of the extensive experience these authors have in the field, editorial limitations did not allow us to fully acknowledge their contribution to the literature. Our interpretation of their work was that whereas they clearly had shown that such an entity as antepartum subdural hematoma exists, it had either not caused death antepartum or alternatively had caused death but was not diagnosed antepartum. Two of their works (references 2 and 5 of their letter) were not available to us at the time of our manuscript preparation. If we misinterpreted any of their earlier work, we apologize.

Stephen Myers, DO

Department of Obstetrics and Gynecology, Mount Sinai Hospital Medical Center, California Ave. at 15th St., Chicago, IL 60608

Unraveling the mysteries of vulval ulceration in human immunodeficiency virus–seropositive women

To the Editors: We believe that the case reported by Covino and McCormack (Covino JM, McCormack WM. Vulval ulcer of unknown etiology in a human immunodeficiency virus–infected woman: response to treatment with zidovudine. Am J Obstet Gynecol 1990;163:116-8) raises several points of interest.

First, although mention is made of oral aphthous ulceration, there is neither further comment on the significance of this nor apparent consideration of other diagnoses such as lichen planus or Crohn's disease. A review of the histopathology of the biopsy specimen with regard to such diagnoses may be worthwhile.

Second, immunocytochemical staining of the biopsy specimen by specific monoclonal antibodies to search for evidence of cytomegalovirus, herpes simplex virus, and human immunodeficiency virus (HIV) infection may have been useful. Cytomegalovirus has been reported as infecting the genital tract in HIV-seropositive women[1] and should be considered as a possible cause of ulceration in this patient. In a case reported from this unit,[2] extensive genital ulceration developed in a 23 year-old HIV-seropositive woman as a result of herpes simplex virus, which was originally sensitive to acyclovir but after 9 months continuous therapy became resistant with clinical deterioration. Zidovudine therapy led to almost complete resolution of this lesion; however, 15 months later a necrotizing proximal myopathy developed and zidovudine therapy ceased. The lesions recurred within 2 weeks, but treatment with intravenous foscarnet was commenced and the lesions completely resolved within 2 weeks. Inasmuch as foscarnet is active against cytomegalovirus, HIV, and herpes simplex virus, we believe that it may be a useful empirical therapy in such cases.

Third, there is no mention of other drugs that this patient may have been taking, for instance prophylaxis against *Pneumocystis carinii* pneumonia. Such a drug history is vital because a fixed drug eruption is an important differential diagnosis in this case.

Fourth, we agree that treatment of genital ulceration in HIV-infected patients is difficult. Zidovudine may have been successful because of its direct antiretroviral activity but also because of a stimulatory effect on the CD4 count. Thalidomide has also been reported to be effective in resistant mucosal ulcers associated with HIV, as have intralesional steroid injections.[3] However, the potential teratogenic effects of the former must be considered before its use in women of child-bearing age.

We believe that a combined team including dermatologists, gynecologists, and genitourinary physicians provides the best management in these difficult cases.

P.D. Kell, MD, and S.E. Barton, MD

Department of Genitourinary Medicine, St. Stephens Clinic, 369 Fulham Road, London, England SW10 9TH

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1. Brown S, Senekjian E, Montag A. Cytomegalovirus infection of the uterine cervix in a patient with acquired immune deficiency syndrome. Obstet Gynecol 1988;71:3:489-91.
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3. Youle M, Hawkins D, Gazzard B. Thalidomide in hyperalgic pharyngeal ulceration in AIDS. Lancet 1990;1:1591.

Reply

To the Editors: We thank Drs. Kell and Barton for their comments regarding our article and for the opportunity to provide additional information regarding our patient.

First, histopathologic examination of the biopsy specimens from the vestibular ulcer disclosed only acute and chronic inflammation, granulation tissue, dysplasia, and condylomatous changes. Biopsy specimens of the oral aphthae were not obtained. There was no evidence of either lichen planus or Crohn's disease.

Second, we agree that immunocytologic staining of the biopsy specimens with specific monoclonal antibodies to search for cytomegalovirus, herpes simplex virus, and human immunodeficiency virus would have been useful. These reagents were not available to us at the time. Multiple specimens obtained from the ulcer were examined for viruses in tissue culture. All were negative for herpes simplex virus and cytomegalovirus. Similarly, histopathologic examination of the biopsy specimens disclosed no inclusions or other evidence of viral infection. Empiric treatment with acyclovir was not associated with healing of the ulcer. Although acyclovir-resistant herpes simplex virus can produce nonhealing genital ulcers, we are confident that our patient did not have herpes simplex virus.

Third, although the patient received brief courses of treatment with a number of medications after the development of the ulcer, she was not receiving medication before the development of the ulcer. Thus we do not believe that she had a fixed drug eruption.

Fourth, we agree that the favorable effect of zidovudine may have been a result of direct antiretroviral activity or of an increase in the effectiveness of the immune system as reflected by the CD4 cell count. However, the ulcer remained healed during the terminal phases of the patient's illness when the CD4 cell count had fallen to 0 cells/mm^3. We did not prescribe thalidomide or intralesional corticosteroids.

Finally, we agree that a multidisciplinary approach is needed for optimal management of difficult problems. We did obtain advice on the management of our patient from experts in many areas including colorectal surgery, gynecologic oncology, immunology, dermatology, and oral surgery.

Jean Marie Covino, RPA, and William M. McCormack, MD
Departments of Medicine and of Obstetrics and Gynecology, State University of New York, Health Science Center at Brooklyn, Box 56, 450 Clarkson Avenue, Brooklyn, NY 11203

Pelvic muscles and episiotomy

To the Editors: After analyzing the data from our research,[1] which involved measurement of the pelvic muscles with a reliable instrument, we question along with Drs. Thorp and Bowes (Thorp JM Jr, Bowes WA Jr. Episiotomy: Can its routine use be defended? Am J Obstet Gynecol 1989;160:1027-30) the routine use of episiotomy.

In our study, subjects were assessed at three intervals (prenatally, 6 to 8 weeks post partum, and 1 year post partum). None of the 22 vaginal birth subjects had episiotomies; 11 had intact perineums, three had first-degree labial lacerations, five had first-degree perineal lacerations, and three had second-degree perineal lacerations. At 1 year post partum ($n = 10$) there was significant improvement of the pelvic muscle function. It was concluded that the restitution of the pelvic muscles may occur over time and therefore episiotomy for prophylactic use in the prevention of pelvic muscle relaxation may not be defensible.

K.R. Cosner, CNM, MSN, Molly C. Dougherty, PhD, and Kevin R. Bishop, ARNP
Box J-197, JHMHC, College of Nursing, Gainesville, FL 32610

REFERENCE

1. Dougherty MC, Abrams R, McKey PL. An instrument to assess the dynamic characteristics of the circumvaginal musculature. Nurs Res 1986;35:202-6.

Reply

To the Editors: We appreciate the interest of Cosner et al. in our article. The data mentioned in their letter are of great interest inasmuch as they report a 1-year follow-up examination. The long-term effect of the method of delivery (including the use of episiotomy) on neuromuscular function of the perineum, rectum, and pelvis is yet to be adequately determined. Only studies with long-term follow-up of the patients will answer this question.

John Thorp, MD, and Watson A. Bowes, Jr., MD
Division of Maternal and Fetal Medicine, Department of Obstetrics and Gynecology, School of Medicine, University of North Carolina at Chapel Hill, CB7570, 214 MacNider Building, Chapel Hill, NC 27599-7570

Books received

A Clinical Guide for the Care of Older Women. Richard L. Byyny and Leon Speroff. 318 pages, illustrated. Baltimore, 1990, Williams & Wilkins. $49.95.

Choosing a Nursing Home. Seth B. Goldsmith. 267 pages. New York, 1990, Prentice Hall Press. $10.95.

Comparative Cardiac Imaging. Edited by Bruce H. Brundage. 573 pages, illustrated. Rockville, Maryland, 1990, Aspen Publishers, Inc. $115.00.

Current Therapy in Obstetrics and Gynecology. Edward J. Quilligan and Frederick P. Zuspan. 454 pages, illustrated. Philadelphia, 1990, W.B. Saunders. $45.00.

Epilepsy: A New Approach. Adrienne Richard and Joel Reiter. 272 pages. New York, 1990, Prentice Hall Press. $17.95.

Foundations for a Feminist Restructuring of the Academic Disciplines. Michele Paludi and Gertrude A. Steuernagal. 276 pages. New York, 1990, Haworth Press, Inc. $24.95.

Journey to Motherhood. Alison Freeland. 199 pages. New York, 1990, Prentice Hall Press. $18.95 (soft cover).

Malpractice Defense: Breast Cancer. Campbell F. Watts. 195 pages, illustrated. Oradell, New Jersey, 1990, Medical Economics Books. $49.95.

Mendelian Inheritance in Man. Victor A. McKusick. 2028 pages. Baltimore, 1990, The Johns Hopkins University Press. $110.00.

Obstetrics. Edited by Sir Alec Turnbull and Geoffrey Chamberlain. 1200 pages, illustrated. Edinburgh, 1989, Churchill Livingstone Inc. $150.00.

A Profile of the Elderly in Argentina. 102 pages, illustrated. Washington, D.C., 1989, Pan American Health Organization (price not listed).

A Profile of the Elderly in Guyana. 124 pages, illustrated. Washington, D.C., 1989, Pan American Health Organization (price not listed).

A Profile of the Elderly in Trinidad and Tobago. 140 pages, illustrated. Washington, D.C., 1989, Pan American Health Organization (price not listed).

Postreproductive Gynecology. Hugh M. Shingleton and W. Glenn Hurt. 590 pages, illustrated. New York, 1990, Churchill Livingstone Inc. $85.00.

State of the Art of the IUD. Edited by H.F.M. van der Pas and Th.O.M. Dieben. 215 pages, illustrated. Dordrecht, The Netherlands: 1989, Kluwer Academic Publishers BV. $70.00.

Stress and Women Physicians. Second Edition. Marjorie A. Bowman and Deborah I. Allen. 216 pages. New York, 1990, Springer-Verlag. $19.50.

Surgical Pathology of the Breast. K. Rogers and A.J. Coup. 149 pages, illustrated. Stoneham, Massachusetts, 1990, Butterworths. $70.00.

Trauma Radiology. Edited by James J. McCort. 483 pages. New York, 1990, Churchill Livingstone Inc. $40.00.

Women's Experience of Breast Feeding. Heather Maclean. 220 pages. Toronto, 1990, University of Toronto Press. $12.95 (soft cover).

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

The Annual International Reproductive Health Seminar, October 24-26, 1991, Key Largo, Florida. Sponsored by IPARC International Population and Reproduction Council, Inc., in cooperation with IPARC Human Development Program, Inc. For further information contact: Howard A. Engle, MD, Director General, IPARC, 975 Arthur Godfrey Road, Suite 102, Miami Beach, FL 33140. Tel.: (305) 531-0047. Outside IPARC North American Region (U.S. and Canada) contact: Arnaldo De Moraes II, MD, PhD, Vice President, IPARC, 20 Princesa Januaria St., Flamengo 22250, Rio de Janeiro, R.J., Brazil. Tel.: (021) 552-7349.

3rd Annual Review Course in Obstetrics and Gynaecology, April 30–May 3, 1991, Delta Chelsea Inn, Toronto, Ontario, Canada. AMA category I study credits. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.

Office Gynecology, April 15-19, 1991, The Hyatt of Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1991 issues from the Publisher, at a cost of $61.00 ($84.00 international) for Vol. 164 (January-June) and Vol. 165 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Subscription Services, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, USA; phone (800) 325-4177, ext. 4351.

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The Women and Infants Hospital of Rhode Island is an Equal Opportunity/Affirmative Action employer and actively solicits applications from minority and protected groups. Interested individuals should send an application and curriculum vitae to:

Marshall Carpenter, MD
Director, Maternal/Fetal Medicine
Chairman, Search Committee
101 Dudley Street
Providence, RI 02905

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Lynnette K. Nieman, M.D.
Chair, Gynecology Search Committee
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National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892

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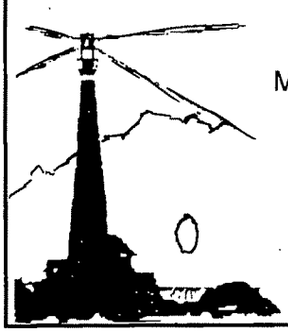
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IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert.**

ADVERSE REACTIONS: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis.

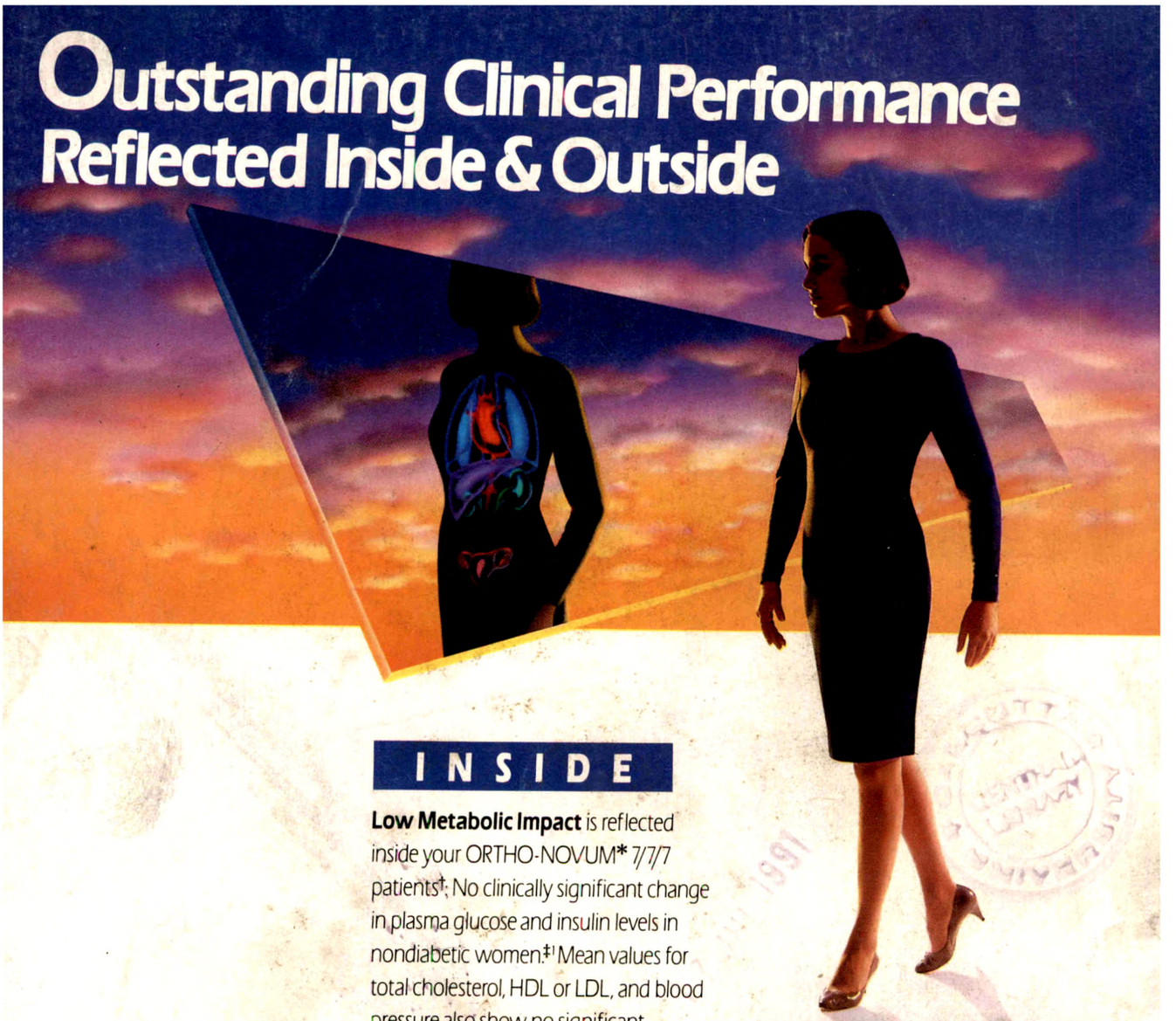
OVERDOSAGE: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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Low incidence of androgenic side effects

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†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

‡Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents.

§An increase in blood pressure has been reported in women taking oral contraceptives.

1. Spellacy WN, Ellingson AB, Tsibris JCM. Glucose and insulin levels after six months of treatment with a triphasic oral contraceptive containing ethinyl estradiol and norethindrone. *J Reprod Med.* 1989;34(8):540-542.

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21- and 28-day regimens

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norethindrone/ethinyl estradiol

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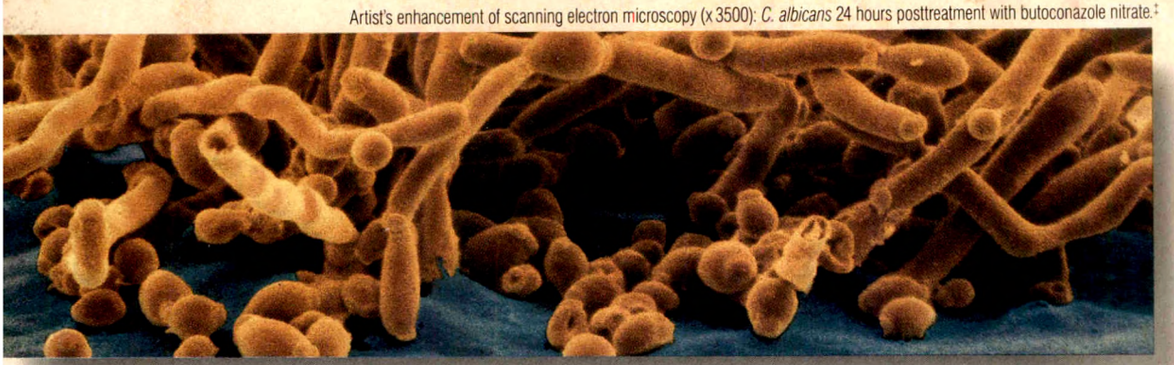
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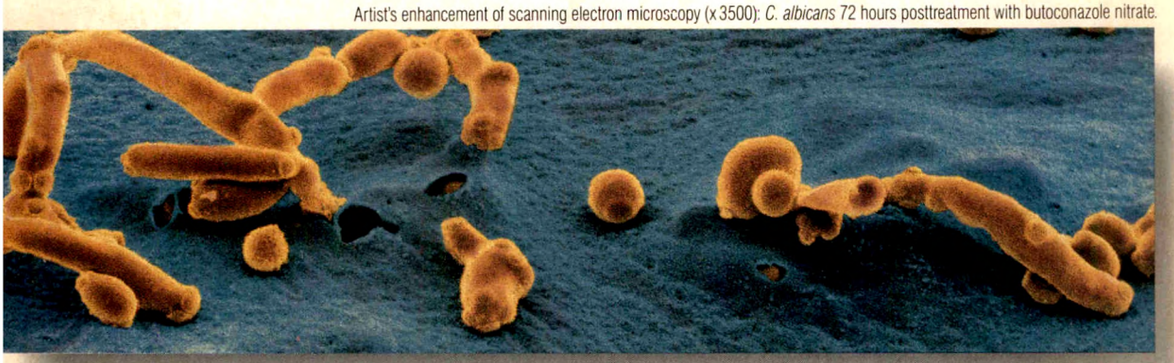
***In vulvovaginal candidiasis,* the proven efficacy of 3-day therapy* with FEMSTAT Prefill means a microbiological cure in 95% of patients.[1†]**

1. Droegemueller W, Adamson DG, Brown D, et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 1984;64:530-534. *In pregnant patients (second and third trimesters only), therapy should be extended to six days. †Microbiological cure in 95% of patients (n = 126) eight days following FEMSTAT 3-day therapy ‡ Micrographs on file, Syntex Laboratories, Inc. Please see next page for brief summary of prescribing information. ©1990 Syntex Laboratories, Inc. 813-H2166-90

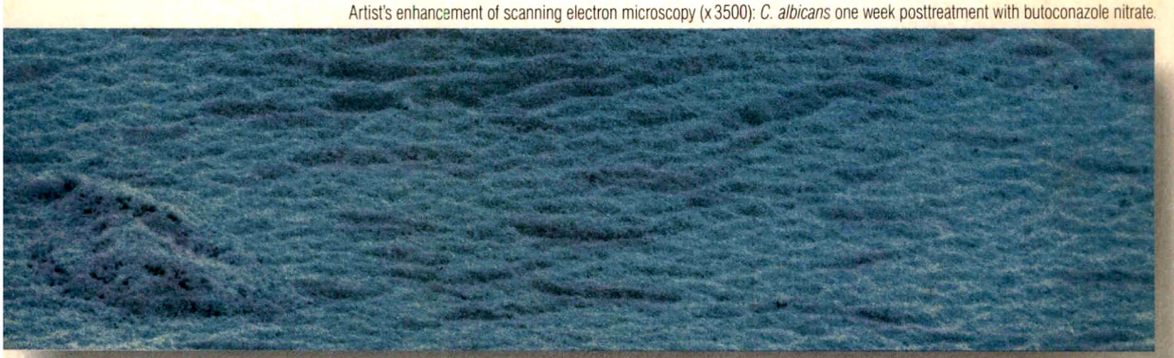
Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 24 hours posttreatment with butoconazole nitrate.‡



Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 72 hours posttreatment with butoconazole nitrate.



Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* one week posttreatment with butoconazole nitrate.



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FEMSTAT PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%



BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

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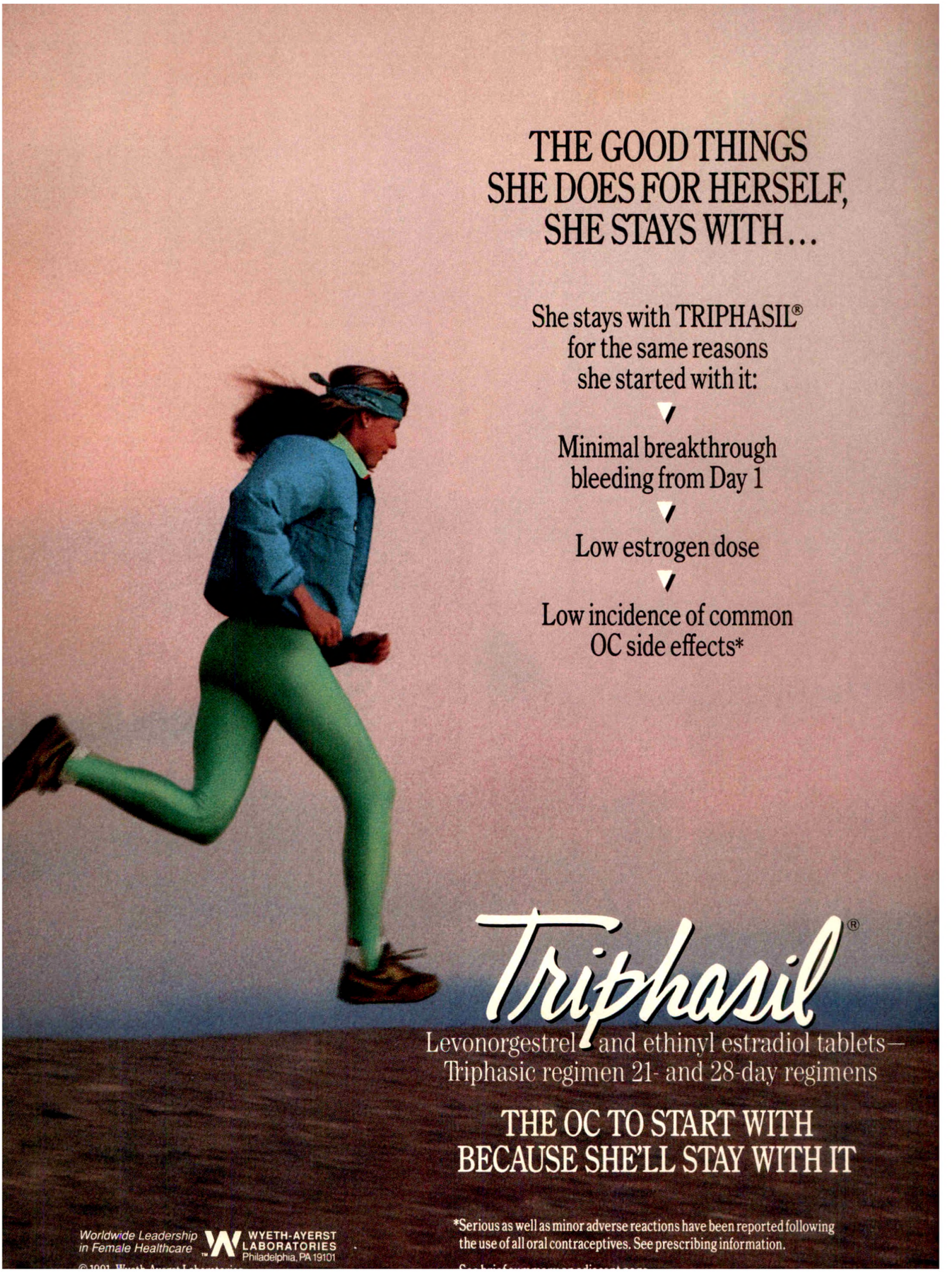
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Indications and Usage — TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications — OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.
The use of oral contraceptives is associated with increased risk of several serious conditions, including thrombo-embolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners pre-scribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS — In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI) — An increased risk of MI associated with the use of OC's has been reported, con-firming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of develcping MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE — In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES — A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34 — 5/100,000; ages 35-44 — 33/100,000; ages 45-49 — 140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS — Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions* — There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma* — Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors* — Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy. Birth Defects in Offspring; and Malignancy in Female Offspring* — Use of female sex hormones — both estrogenic and progestational agents — during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease* — Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects* — Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure* — Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache* — Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities* — Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy* — Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding* — OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions — GENERAL — 1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear. 2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related. 4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined. 8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's. a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient — See Patient Package Labeling.

Drug Interactions — Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis — See Warnings section for information on carcinogenesis.

Pregnancy — Category X. See Contraindications, Warnings.

Nursing Mothers — See Warnings.

Adverse Reactions — An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose — Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration — For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)
Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days. For full details on dosage and administration see prescribing information in package insert.

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April
1991

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Bruce A. Cunningham, PhD

New York, New York

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Miami Beach, Florida

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San Antonio, Texas

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Richard E. Besinger, MD, Jennifer R. Niebyl, MD, William G. Keyes, MD, PhD, and Timothy R.B. Johnson, MD

Baltimore, Maryland

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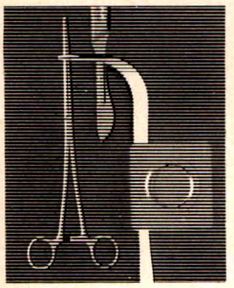
(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

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Count on

CEFOTAN® IM/IV

(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary tract infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).
Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli.*
Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli.*
Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).
Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).
Bone and joint infections caused by *Staphylococcus aureus.**
*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.

PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be

developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).
Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).
Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).
Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).
Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:} \quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.

1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

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The induction of premature luteolysis in normal women—follicular phase luteinizing hormone secretion and corpus luteum function in the subsequent cycle 989

Michael R. Soules, MD, William J. Bremner, MD, PhD, Kristine D. Dahl, PhD, Jean E. Rivier, PhD, Wylie W. Vale, PhD, and Donald K. Clifton, PhD

Seattle, Washington, and La Jolla, California

Decreased luteal progesterone levels induced by an antagonist to gonadotropin-releasing hormone did not lead to an increased frequency of luteinizing hormone pulses or luteal phase deficiency in the subsequent cycle.

Assessment of current International Federation of Gynecology and Obstetrics staging of vulvar carcinoma relative to prognostic factors for survival (A Gynecologic Oncology Group Study) 997

Howard D. Homesley, MD, Brian N. Bundy, PhD, Alexander Sedlis, MD, Edgardo Yordan, MD, Jonathan S. Berek, MD, Antoine Jahshan, MD, and Rodrique Mortel, MD

Winston-Salem, North Carolina, Buffalo and Brooklyn, New York, Chicago, Illinois, Los Angeles, California, and Philadelphia and Hershey, Pennsylvania

Further refinements are warranted within stage III in the current International Federation of Gynecology and Obstetrics vulvar carcinoma staging system, which is based on analysis of the independent prognostic factors of groin node status and lesion diameter.

Comparison of pulsatile and continuous ritodrine administration: Effects on uterine contractility and the β-adrenergic receptor cascade 1005

Steve N. Caritis, MD, Jye Ping Chiao, MS, and Pam Kridgen, BS

Pittsburgh, Pennsylvania

Administration of identical doses of ritodrine for 24 hours by continuous infusion or by intermittent pulses produces different effects on myometrial β-adrenergic recptors, yet oxytocin-induced contractility is inhibited for 24 hours by both regimens.

Rapid intramembranous absorption into the fetal circulation of arginine vasopressin injected intraamniotically 1013

William M. Gilbert, MD, Cecilia Y. Cheung, PhD, and Robert A. Brace, PhD

San Diego, California

Intraamniotically injected arginine vasopressin appears rapidly in fetal plasma independent of fetal swallowing, suggesting the intramembranous pathway is primarily involved in the regulation of amniotic fluid volume and composition.

Effect of intravenous cocaine on uterine blood flow in the gravid baboon 1021

Mark A. Morgan, MD, Susan L. Silavin, PhD, Mildred Randolph, DVM, Gerald G. Payne, Jr., MD, Roger E. Sheldon, MD, John I. Fishburne, Jr., MD, Richard A. Wentworth, PhD, and Peter W. Nathanielsz, MD, PhD

Oklahoma City, Oklahoma, and Ithaca, New York

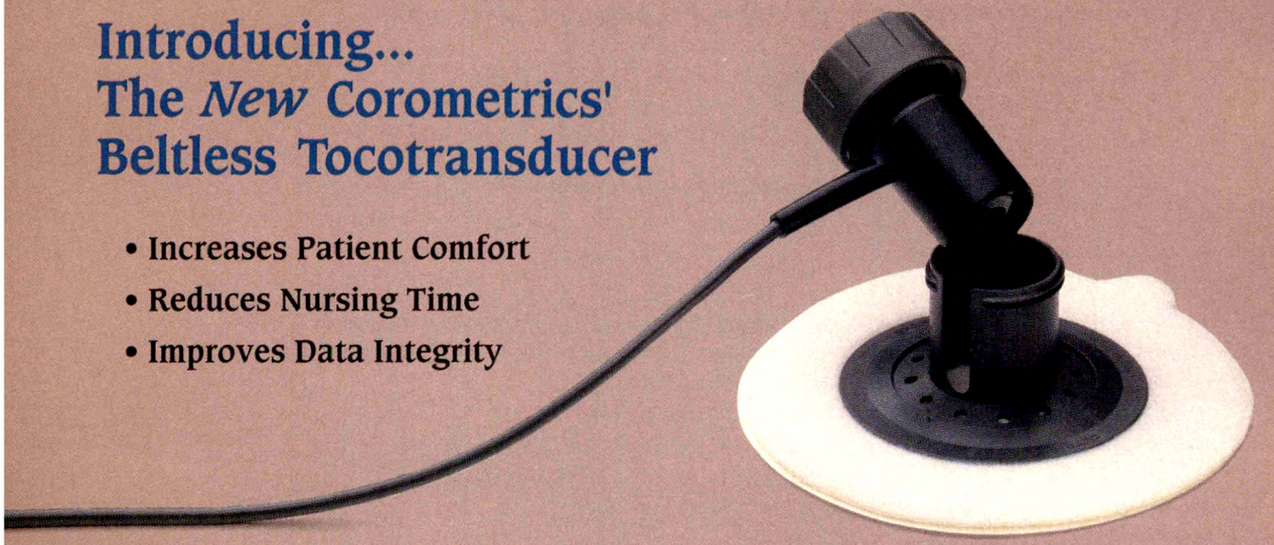
Low-dose intravenous cocaine infusions reduce uterine blood flow in a dose-dependent manner when administered to the unanesthetized, chronically instrumented, pregnant baboon.

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c-*myc* Protooncogene polypeptide expression in endometriosis 1031
Robert S. Schenken, MD, Julia V. Johnson, MD, and Robert M. Riehl, PhD
San Antonio, Texas

The c-*myc* protooncogene polypeptide was identified in glandular nuclei and cytoplasm and in stromal nuclei of endometriotic tissue obtained during the proliferative phase of the menstrual cycle.

Serum interleukin-6 levels correlate with disease status in patients with epithelial ovarian cancer 1038
Jonathan S. Berek, MD, Catherine Chung, BS, Klara Kaldi, BS, Joanna M. Watson, BSc, Reba M. Knox, MS, and Otoniel Martinez-Maza, PhD
Los Angeles, California

Serum interleukin-6 levels were elevated in the majority of patients with bulky residual epithelial ovarian cancer but not in most patients with microscopic disease.

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Ultrasonography is of limited value in screening for congenital malformation.

The current status of maternal and fetal blood flow velocimetry J.A. Low, MD *Kingston, Ontario,Canada* 1049
Doppler assessment of maternal and fetal blood flow velocity should be limited to the investigational setting.

Ultrasonography in the diagnosis of gynecologic disorders J. Robert Willson, MD
Albuquerque, New Mexico 1064
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Stephen H. Cruikshank, MD
Minneapolis, Minnesota

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Sherman Elias, MD, George J. Annas, JD, MPH, and Joe Leigh Simpson, MD
Memphis, Tennessee, and Boston, Massachusetts

Although population screening for cystic fibrosis carrier status offers promising results, only individuals or couples with a family history of cystic fibrosis should be offered screening at this time.

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Determinants of perinatal outcome in pregnancy-induced hypertension with absence of umbilical artery end-diastolic frequencies 1084
Fiona M. Fairlie, MD, Michael Moretti, MD, James J. Walker, MD, and Baha M. Sibai, MD
Glasgow, Scotland, and Memphis, Tennessee

In pregnancy-induced hypertension with absence or reversal of umbilical artery end-diastolic frequencies, the most important determinant of perinatal outcome was gestational age when hypertension occurred.

Intrauterine transfusion treatment of nonimmune hydrops fetalis secondary to human parvovirus B19 infection 1090
Vicken Sahakian, MD, Carl P. Weiner, MD, Stanley J. Naides, MD, Roger A. Williamson, MD, and Laura L. Scharosch, AB
Iowa City, Iowa

Hydrops that is secondary to parvovirus may be treated.

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Mark I. Evans, MD, Arie Drugan, MD, Sidney F. Bottoms, MD, Lawrence D. Platt, MD, Charles A. Rodeck, MD, Manfred Hansmann, MD, and John C. Fletcher, PhD
Detroit, Michigan, Haifa, Israel, Los Angeles, California, London, England, Bonn, Federal Republic of Germany, and Charlottesville, Virginia

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Albert P. Sarno, Jr., MD, Major, MC, USA
Los Angeles, California, and Honolulu, Hawaii

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Susan L. Hendrix, DO, and Harold J. Sauer, MD
Lansing and East Lansing, Michigan

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David M. Sherer, MD, Jacques S. Abramowicz, MD, Mary Lou D'Amico, RN, Christine B. Caverly, RN, and James R. Woods, Jr., MD
Rochester, New York

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Kazuko Moroi, MD, Siu Saito, MD, Takeshi Kurata, MD, PhD, Tetsutaro Sata, MD, and Masahiko Yanagida, MD
Tokyo, Japan, and Bethesda, Maryland

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Steven A. Narod, MD, Silvia de Sanjosé, MD, PhD, and Cesar Victora, MD, PhD
Lyon, France, and Pelotas, Brazil

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Toru Takahashi, MD, Beth Marcus, BA, Richard G. Scheerer, MD, and Michael Katz, MD
Kawasaki, Japan, and San Francisco, California

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Klaus G. Schmidt, MD, Mariarosaria Di Tommaso, MD, Norman H. Silverman, MD, and Abraham M. Rudolph, MD
San Francisco, California

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Immunohistochemical localization of gonadotropin-releasing hormone during implantation in the New Zealand White rabbit 1127
Paul A. Bergh, MD, Ted L. Anderson, PhD, and Glen E. Hofmann, MD, PhD
New York, New York, and Nashville, Tennessee

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Roger C. Young, MD, PhD, Lea Herndon Smith, and Nels C. Anderson, Jr., PhD
Durham, North Carolina, and Charleston, South Carolina

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Ernest M. Graham, MD, Aemil J.M. Peters, Robert M. Abrams, PhD, Kenneth J. Gerhardt, PhD, and David J. Burchfield, MD
Gainesville, Florida

Sound pressure levels in the abdomen of sheep vary with applied static force and frequency of vibration on abdominal surface.

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Dinesh M. Shah, MD, Kazumi Higuchi, MD, PhD, Tadashi Inagami, PhD, and Kevin G. Osteen, PhD
San Antonio, Texas, and Nashville, Tennessee

Endometrial stromal cells in culture secrete high amounts of active renin compared with mesenchymal cell cultures, and progesterone stimulates further active renin secretion.

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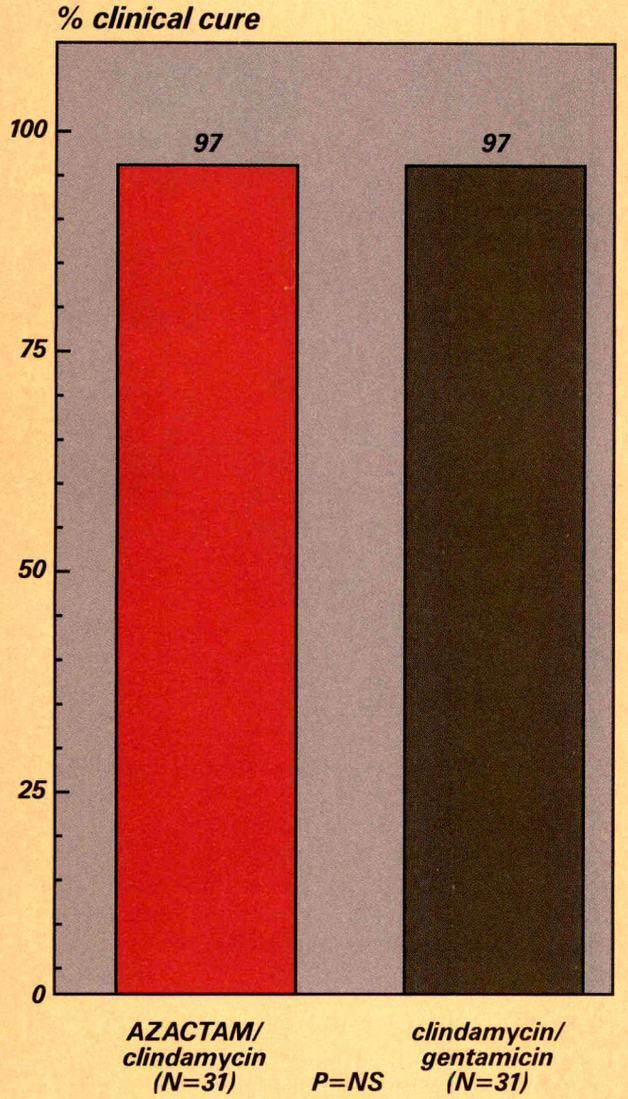
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IN POSTPARTUM ENDOMETRITIS*



A randomized, comparative trial of AZACTAM versus gentamicin, each with clindamycin, for the treatment of endometritis after delivery.[1]

No universal criteria exist that may satisfy a single definition of "cure." Use of this term may vary between clinical settings and investigators, and the reader is urged to consult the reference listed.

Azactam®
aztreonam
delivers...

*Due to susceptible organisms.

†Although a useful guide, in vitro data do not necessarily correlate with therapeutic outcome.

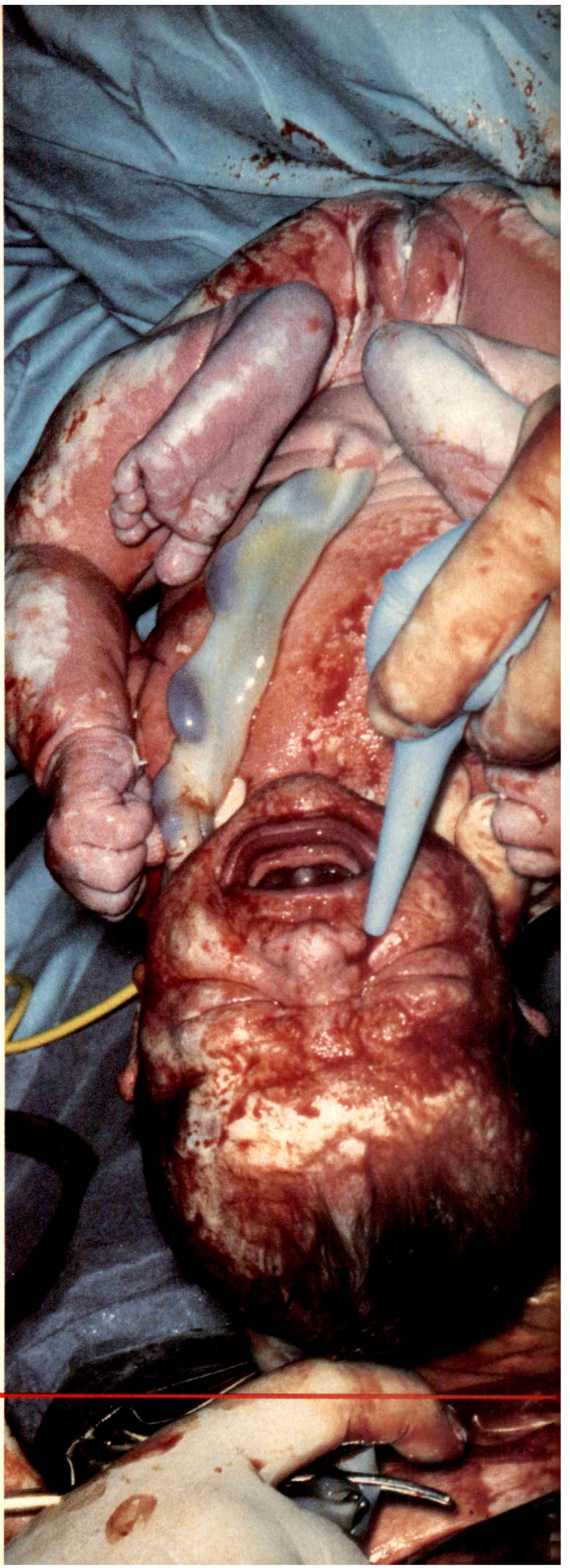
‡Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. See full prescribing information for recommendations on patients with immediate hypersensitivity reactions to penicillins.

Following IV administration, the most commonly seen adverse reactions occurred in less than 2% of patients, including local reactions, diarrhea, nausea/vomiting, and rash.

more than you're expecting...

- *Broad aerobic gram-negative in vitro coverage, including Escherichia coli, Proteus mirabilis, and Enterobacter cloacae.†*
- *Greater than 90% cure rates in combination with clindamycin.[1-3]*
- *No time-consuming, costly, routine therapeutic drug monitoring.*
- *May be admixed in the same bag with clindamycin.*
- *May be used in penicillin-allergic patients.‡*
- *Pregnancy Category B.*

There are no adequate and well-controlled studies in pregnant women. AZACTAM should be used in pregnant women only if clearly needed.



Azactam®
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A logical alternative to aminoglycosides.

Please see adjacent page for brief summary of prescribing information.

Azactam®
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AZACTAM® FOR INJECTION
Aztreonam For Injection

DESCRIPTION–AZACTAM (aztreonam, Squibb) is the first member of a new class of antibiotics classified as monobactams. AZACTAM is a totally synthetic bactericidal antibiotic with activity against a wide spectrum of gram-negative aerobic pathogens. The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics.

AZACTAM For Injection is a sterile, nonpyrogenic, sodium-free, white to yellowish-white lyophilized cake, containing approximately 780 mg arginine per gram of aztreonam for intramuscular or intravenous use following constitution. Aqueous solutions of the product have a pH in the range of 4.5-7.5.

INDICATIONS AND USAGE–Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: **Urinary Tract Infections** (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca*, Citrobacter* species* and *Serratia marcescens**. **Lower Respiratory Tract Infections,** including pneumonia and bronchitis caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens**. **Septicemia** caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*, Serratia marcescens** and *Enterobacter* species. **Skin and Skin-Structure Infections,** including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Citrobacter* species*. **Intra-abdominal Infections,** including peritonitis caused by *Escherichia coli, Klebsiella* species, including *K. pneumoniae, Enterobacter* species including *E. cloacae*, Pseudomonas aeruginosa, Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**. **Gynecologic Infections,** including endometritis and pelvic cellulitis caused by *Escherichia coli, Klebsiella pneumoniae*, Enterobacter* species* including *E. cloacae*,* and *Proteus mirabilis**.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy–Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organisms(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an antianaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS–Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS–Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin). Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

PRECAUTIONS–General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility–Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy–Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers–Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use–Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS–Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity; *Hypersensitivity*–anaphylaxis. *Hematologic*–pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. *Gastrointestinal*–abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). *Dermatologic*–purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. *Cardiovascular*–hypotension, transient ECG changes (ventricular bigeminy and PVC). *Respiratory*–one patient experienced flushing, chest pain, and dyspnea. *Hepatobiliary*–hepatitis, jaundice. *Nervous System*–seizure, confusion, vertigo, paresthesia, insomnia, dizziness. *Musculoskeletal*–muscular aches. *Special Senses*–tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. *Other*–vaginal candidiasis, vaginitis, breast tenderness. *Body as a Whole*–weakness, headache, fever, malaise.

Adverse Laboratory Changes–Those reported without regard to drug relationship during clinical trials were: *Hepatic*–elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*–increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Renal*–increases in serum creatinine.

OVERDOSAGE–If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION–Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg,** or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231C)

References: ***1.*** *Greenberg RN, Reilly PM, Weinandt WJ, et al: Comparison trial of clindamycin with aztreonam or gentamicin in the treatment of postpartum endometritis. Clin Ther 10(1):36-39, 1987.* ***2.*** *Gibbs RS, Blanco JD, Lipscomb KA, et al: Aztreonam versus gentamicin, each with clindamycin, in the treatment of endometritis. Obstet Gynecol 65:825-829, 1985.* ***3.*** *Henry SA: Overall clinical experience with aztreonam in the treatment of obstetric-gynecologic infections. Rev Infect Dis 7(suppl 4):S703-S708, 1985.*

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2. **Dr. Quilligan**—manuscripts from states west of Pennsylvania, West Virginia, Kentucky, Tennessee, and Mississippi; manuscripts from Hawaii, Alaska, Canada, and abroad (except England, Israel, and Italy). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. *Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.*

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General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of glossy prints of illustrations are required.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, figure legends, and tables.

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Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare

Estimating length of manuscripts

With the exception of Current Development articles, the length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

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BOOKS: Ledger WJ. Dystocia and prolonged labor. In: Wilson JR, Carrington ER, eds. Obstetrics and gynecology. St. Louis: CV Mosby, 1987 vol 8:474-93.

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—All pages are numbered in the following order: title page, condensation, abstract, body of the text, acknowledgments only of persons who have made substantive contributions to the study, references, legends, and tables.
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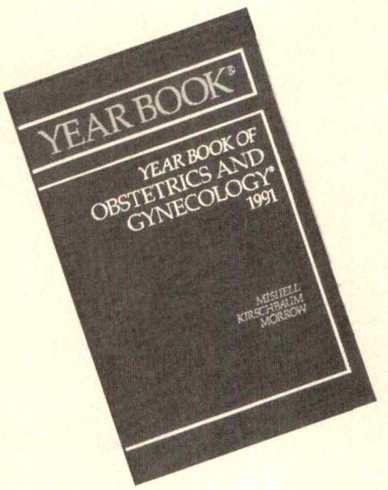
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TRI·LEVLEN® Levonorgestrel and ethinyl estradiol tablets–Triphasic regimen

TRI-LEVLEN® 28 TRI-LEVLEN® 21

BRIEF SUMMARY

Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

Contraindications

Oral contraceptives should not be used in women with any of the following conditions: thrombophlebitis or thromboembolic disorders, a past history of deep-vein thrombophlebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estrogen-dependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pill use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long-term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of disease, namely, a ratio of the incidence of a disease among oral-contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral-contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods.

1. *Thromboembolic Disorders and Other Vascular Problems*

a. *Myocardial Infarction.* An increased risk of myocardial infarction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral-contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. *Thromboembolism.* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to four-fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

c. *Cerebrovascular diseases.* Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and nonusers, for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

d. *Dose-related risk of vascular disease from oral contraceptives.* A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogen used in the contraceptive. The amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral-contraceptive agents should be started on preparations containing less than 50 mcg of estrogen.

e. *Persistence of risk of vascular disease.* There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estrogens.

2. *Estimates of Mortality from Contraceptive Use.* One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral-contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral-contraceptive users is based on data gathered in the 1970's–but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with oral-contraceptive use after age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. Therefore, the Committee recommended that the benefits of oral-contraceptive use by healthy nonsmoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. *Carcinoma of the Reproductive Organs.* Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonusers and differences in age at start of use, has been questioned. Some studies suggest that oral-contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. *Hepatic Neoplasia.* Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users.

5. *Ocular Lesions.* There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. *Oral-Contraceptive Use Before or During Early Pregnancy.* Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing oral-contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is confirmed.

7. *Gallbladder Disease.* Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. *Carbohydrate and Lipid Metabolic Effects.* Oral contraceptives have been shown to cause glucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hyperinsulinism, while lower doses of estrogen cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see "Warnings" 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral-contraceptive users.

9. *Elevated Blood Pressure.* An increase in blood pressure has been reported in women taking oral contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestogens. Women with a history of hypertension or hypertension-related diseases, or renal disease should be encouraged to use another method of contraception. If women with hypertension elect to use oral contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension among ever-and never-users.

10. *Headache.* The onset or exacerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. *Bleeding Irregularities.* Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestogen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

Precautions

1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. (See "Warnings" 1d.) 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine-and liver-function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral-contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See "Warnings" section. 10. PREGNANCY. Pregnancy Category X. See "Contraindications" and "Warnings" sections. 11. NURSING MOTHERS. Small amounts of oral-contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

Information for the Patient

See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see "Warnings" section): thrombophlebitis, arterial thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, gallbladder disease, hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; temporary infertility after discontinuation of treatment; edema; melasma which may persist; breast changes: tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, hemolytic uremic syndrome, Budd-Chiari syndrome, acne, changes in libido, colitis, sickle-cell disease.

Overdosage

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits

The following noncontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenorrhea.

Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer.

Dosage and Administration

To achieve maximum contraceptive effectiveness, TRI-LEVLEN® tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours.

For full details on dosage and administration see prescribing information in package insert. 60627-0 5/90

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Cell adhesion molecules and the regulation of development

Bruce A. Cunningham, PhD

New York, New York

Cell adhesion molecules, in conjunction with the other morphoregulatory molecules, substrate adhesion molecules and cell junctional molecules, are dynamically expressed in coordinate patterns throughout development. Their activities are linked to a variety of cellular processes, and their ability to influence mechanochemical processes allows them to influence a variety of other fundamental developmental events. The clinical significance of these molecules remains to be determined, but they are clearly involved in a number of pathologic conditions and could become the focus of a wide range of diagnostic techniques and eventually even therapeutic designs. (AM J OBSTET GYNECOL 1991;164:939-48.)

Key words: Structural families, expression pattern, perturbation studies, cellular transfection, cancer and other diseases

Over the past decade a variety of new techniques has opened previously inaccessible arenas to molecular and cellular analyses. Among these arenas are the fundamental processes in embryonic development, including cell adhesion and migration. The importance of cell-cell interactions to developmental events has long been noted,[1, 2] but the recent identification and characterization of glycoproteins that mediate such interactions have dramatically altered earlier speculations about the nature of cell adhesion molecules and their role in embryogenesis.[3-5] These glycoproteins not only mediate interactions between cells but are dynamically expressed at critical times and places where they can act as key regulators of developmental events. Because they can affect cell patterns and tissue signaling through mechanochemical effects on cell linkage, shape, and movement, they have been termed morphoregulatory molecules.[6]

Morphoregulatory molecules (Fig. 1) include not only cell adhesion molecules (Fig. 1, *A*) but also substrate adhesion molecules and their receptors (e.g., integrins) and cell junctional molecules. A review of substrate adhesion molecules and cell junctional molecules is beyond the scope of this discussion, but some mention should be made of their relationship to cell adhesion molecules.

Substrate adhesion molecules (Fig. 1, *B*) are a diverse group of extracellular molecules that include collagens, glycoproteins, and proteoglycans.[7-9] They differ from the other morphoregulatory molecules in that they are not necessarily made by the cells that bind them. Substrate adhesion molecules can bind to cells through a number of different kinds of receptors; they can also bind to each other and can influence each others' activities. Some, such as cytotactin-tenascin,[10, 11] can influence cells in unusual ways. For example, neural crest cells will not migrate on cytotactin substrates,[12, 13] and when fibroblasts bind to cytotactin-coated substrates, they will not flatten but will remain round.[14] Such negative activities are probably critical to the mechanisms and timing of a number of developmental processes. The ability of cells to migrate on substrate adhesion molecules appears to be inversely related to their ability to interact with each other in that cell adhesion molecules are down regulated on migrating cells.[15, 16] It is becoming increasingly clear, however, that some cell adhesion molecules are found in excreted or soluble forms that may act as substrate adhesion molecules.[17, 18].

From the Rockefeller University.

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Reprint requests: Bruce A. Cunningham, PhD, The Rockefeller University, New York, NY 10021.

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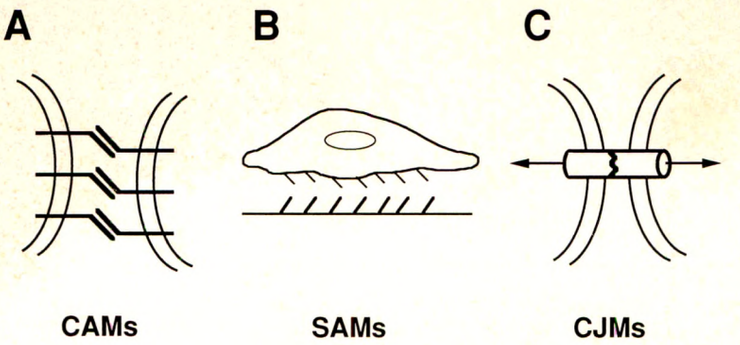


Fig. 1. Morphoregulatory molecules[6] can affect cell patterning events and tissue signaling via mechanical-chemical effects on cell-cell interactions, cell movement, and cell shape.[3] **A**, Cell adhesion molecules *(CAMs bent lines)* are firmly attached to cell surface *(arced double line)* and link cells specifically to each other. **B**, Substrate adhesion molecules *(SAMs, thick, upward-slanted lines)* are found in extracellular matrix *(horizontal line)* and bind to cells via specific receptors *(downward-slanted lines)* on cell surface. **C**, Cell junctions are usually complex structures that include cell junctional molecules *(CJMs)*. Junctions can provide tight seals (tight junctions), communicating links between cells (gap junctions), or attachments to filamentous structure within the cell (adherens junctions and desmosomes).

Cell junctions (Fig. 1, *C*) are relatively elaborate connections between cells that can involve many molecules.[19] They provide seals between cells, allow cells to communicate by transfer of small molecules, and link the cell surfaces with filamentous structures within the cell. The formation of junctions appears to require a prior cell adhesion molecule–mediated event,[20] and some junctions contain cell adhesion molecules or molecules that resemble cell adhesion molecules. For example, adherens junctions can include at least two cell adhesion molecules, A-CAM (adherens junction–specific cell adhesion molecule) (N-cadherin)[21] and L-CAM (uvomorulin),[22] and desmosomes[23] include proteins that are homologous to these calcium-dependent cell adhesion molecules.

Cell adhesion molecules

Cell adhesion molecules (Fig. 1, *A*) are cell surface glycoproteins that either extend through the cell membrane or are attached to the membrane by a lipid anchor. Contrary to earlier notions, there appear to be relatively few kinds of cell adhesion molecules, and a specific molecule can be expressed at a variety of times and places throughout development. This means that when and where a cell adhesion molecule is expressed are as crucial to developmental events as its binding specificity. In addition, specific cell adhesion molecules that are expressed on early embryonic cells are also expressed in a variety of adult tissues. This observation altered previous notions that specific recognition molecules might be expressed only for short periods during development; it also meant that cell adhesion molecules could be isolated from late embryonic or even adult tissues and used for direct biochemical and functional studies.

Different cell adhesion molecules differ in the times at which they are expressed and in the levels of their expression. They can be loosely classified as primary or secondary.[24] Primary cell adhesion molecules such as the neural molecule (N-CAM) and the liver molecule (L-CAM) appear on the earliest embryonic cells and on derivatives of all three germ layers.[15, 16, 25] N-CAM, for example, is seen at least transiently in muscle, heart, liver, kidney, pancreas, lung, and intestine, whereas L-CAM is seen on nearly all epithelia. Secondary cell adhesion molecules such as the neuron-glia molecule (Ng-CAM)[26] appear late in development (after neurulation) and have a highly restricted distribution (postmitotic neurons and Schwann cells). It was anticipated that there would be many more secondary than primary cell adhesion molecules[24] and this notion is gradually being substantiated. N-CAM[27] and L-CAM[28] bind cells by a homophilic mechanism, i.e., a cell adhesion molecule on one cell binds the same one on another. Ng-CAM binds neurons to each other by a homophilic mechanism and neurons to glia by a heterophilic mechanism.[29]

Analyses of their structures have indicated that most cell adhesion molecules can be sorted into one of two families (Fig. 2). Members of one of these families (Fig. 2, *A*) have structural features like those found in antibodies (immunoglobulins) and most closely resemble N-CAM.[30] Ng-CAM, for example, is a member of this family.[31] Members of the other group (Fig. 2, *B*) are more closely related molecules that require calcium for their activity; these calcium-dependent cell adhesion molecules, or cadherins,[4] all resemble L-CAM.[32] The structural similarities among cell adhesion molecules in these two families are greatly aiding efforts to correlate their structure with their functions and in

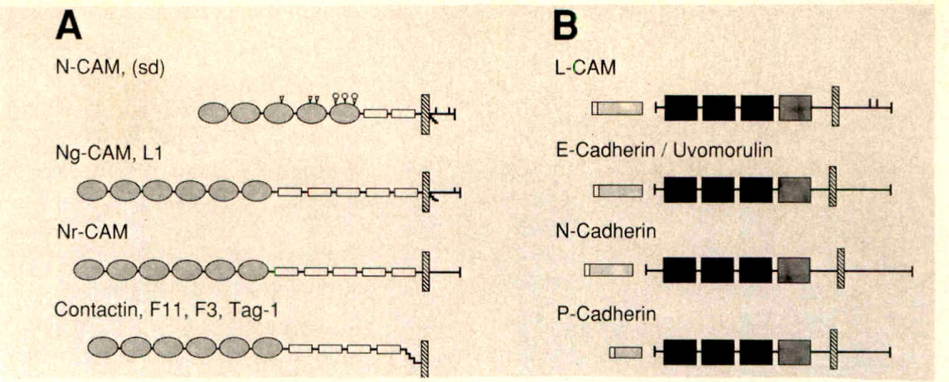


Fig. 2. Structural features of two families of cell adhesion molecules. **A**, All members of the N-CAM family contain immunoglobulin-like domains *(ovals)* and some contain segments resembling fibronectin type III repeats *(rectangles)*. Cell adhesion molecules can extend across the cell membrane *(vertical hatched rectangle)* or attach to the membrane via a lipid linker *(stairstep symbol)*. Included on the model of the most widely distributed form (small cytoplasmic domain, *sd*) of N-CAM[30] are indications of posttranslational modifications: *inverted triangles*, N-linked carbohydrates; *small circles*, polysialic acid, which is unique to N-CAM; and *vertical lines*, sites of phosphorylation on serine or thyronine. The related neural cell adhesion molecules shown are chicken Ng-CAM,[31] mouse L1,[134] chicken Nr-CAM,[135] chicken contactin/F11,[136, 137] mouse F3,[138] and rat TAG-1.[18] **B,** Members of L-CAM family are closely related molecules that require calcium for their activity. They are each made as a larger precursor with a signal sequence *(small open box)* and about 100 amino acids *(thin stippled rectangle)* that are cleaved off before expression on the cell surface. They contain internal segments that are similar to each other *(shaded boxes)*; those shown are all transmembrane *(vertical stippled rectangle)* proteins. Despite their similarities, they have different specificities and tissue distributions; L-CAM[32] and its mammalian equivalent E-cadherin[67] or uvomorulin[66] are found in early embryos and epithelia, whereas N-cadherin[71] is found in brain, lens, and heart and P-cadherin[72] is detected in placenta and endodermal tissues.

some cases[33] have led to the demonstration that molecules having such structures, but having no previously known function (e.g., carcinoembryonic antigen), can act as cell adhesion molecules.

Neural cell adhesion molecule. N-CAM was the first cell adhesion molecule to be isolated[34, 35] and characterized.[30] It has a number of unusual features, including long chains of covalently attached α2,8-linked polysialic acid.[36, 37] This negatively charged sugar is present in largest amounts (30 gm/100 gm protein) in N-CAM from embryonic tissues and decreases to 30% of that value in most adult tissues. The sugar is not directly involved in binding[38] but influences binding so that embryonic forms of N-CAM bind at slower rates than adult forms.[27]

N-CAM (Fig. 2, *A*) has a large extracellular domain that includes five continuous segments that resemble each other and the domains of immunoglobulins that are followed by two segments resembling the type III repeats in fibronectin.[30, 39] The binding region is apparently within the first four immunoglobulin-like domains,[38] whereas the fifth contains the polysialic acid.[40] There are multiple forms of the molecule that differ in the size of their cytoplasmic domains or in the mode of attachment to the membrane.[41, 42] The most widely distributed small cytoplasmic domain form is shown in Fig. 2, *A*. The larger, or large cytoplasmic domain form has a cytoplasmic domain that contains 261 more amino acids than the small cytoplasmic domain form. The third basic form, small surface domain, has no cytoplasmic domain but is attached to the membrane through a phosphatidylinositol linker. Soluble forms of N-CAM are also seen,[43, 44] and there are other variants with small inserts between the fibronectin repeats[17, 44-46] or in the fourth immunoglobulin domain.[47, 48]

All forms of N-CAM appear to undergo the embryonic to adult transition in the sialic acid component, and the individual forms are differently expressed. The large cytoplasmic domain form is seen only in neural tissues,[49, 50] whereas the small surface domain form is seen in muscle, glia, and other specialized locations.[41, 43, 46] The small cytoplasmic domain form is seen in varying amounts in nearly all tissues where N-CAM is expressed.

All forms of N-CAM are derived from a single gene through differential ribonucleic acid splicing.[48, 49] The gene in chickens is large (>70 kb) and has more than 20 exons.[51] The gene has been mapped to mouse chromosome 9[52] and human chromosome 11[53] near a region where a number of translocations are seen.

N-CAM family. Since the description of the structure of N-CAM, a number of cell surface proteins have been described that have many features in common with

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each other and with N-CAM. Some of those found in the nervous system are compared with N-CAM in Fig. 2, *A*. The distinguishing feature is the presence of one or more Ig-like domains that resemble those in N-CAM more closely than those in other immunoglobulin-related molecules (e.g., immunoglobulins, transplantation antigens, β_2-microglobulin). A number of neural cell adhesion molecules belong to this family and most of these also have multiple fibronectin repeats. Other members of the family include those from the immune system,[54, 55] PE-CAM (endo-CAM) found on endothelial cells,[56] and the opioid binding protein OB-CAM.[57] In addition, some members of the N-CAM family are viral receptors,[58] including the poliovirus receptor, CD4 (the human immunodeficiency virus receptor), and I-CAM1 (the rhinovirus receptor).

The resemblance of N-CAM to immunoglobulins, the transplantation antigens, and other members of the immunoglobulin superfamily prompted the suggestion that the evolutionary precursor of N-CAM gave rise to the immune system.[59] Convincing support for this hypothesis has come from the identification of N-CAM-like molecules in the nervous systems of insects,[60-62] which have no adaptive immune system.

L-CAM and related molecules. L-CAM was first detected in chicken liver.[63, 64] Comparable molecules were also found in mammals, including humans,[65] and were given various names; the most commonly used names for these are uvomorulin[66] and E-cadherin.[67] All are assumed to be the same molecule in different species, so here, for simplicity, I generally refer to all of these as L-CAM. L-CAM[32] is a large transmembrane glycoprotein with apparent internal repeats (Fig. 2, *B*), but it does not resemble immunoglobulins, N-CAM, or fibronectin; it has no polysialic acid and is expressed in only a single form. It binds calcium and requires calcium for its homophilic binding activity and biochemical integrity.

The gene for L-CAM[68] is small (10 kb) and complex (>16 exons), but there is no evidence for differential splicing. In mice it is located on chromosome 8[69] and in humans on chromosome 16.[70]

A variety of other calcium-dependent cell adhesion molecules, or cadherins, have been described.[4] They include N-cadherin[71] or A-CAM[21] found in the nervous system, heart, and lens; P-cadherin,[72] found in placenta and endodermal cells; and more recently T-cadherin,[73] from the nervous system, and V-cadherin,[74] from endothelial cells. L-CAM, P-cadherin, and N-cadherin (Fig. 2, *B*) are 50% to 80% identical in amino acid sequence but have different binding specificities and different tissue distributions; the gene for N-cadherin is on a different human chromosome (chromosome 18) than that for L-CAM.[75]

Expression of cell adhesion molecules

An outstanding feature of cell adhesion molecules is their dynamic pattern of expression at critical sites throughout development. These observations alone are sufficient to indicate their active role in development. Some primary cell adhesion molecules are detected either in oocytes or as early as the two-cell stage.[76, 77] On mouse blastocysts L-CAM (uvomorulin) is essential for compaction,[78] and N-CAM and L-CAM are expressed on all cells at early stages, at least in frog and chicken embryos.[15, 16, 25, 77] When cells migrate through the primitive streak to form middle layers or mesoderm, N-CAM is down regulated but is often expressed when mesenchymal cells condense to form epithelia. Later, N-CAM (but not L-CAM) is seen on cells of the neural plate and L-CAM is expressed by itself or together with N-CAM in epithelia that will give rise to skin (ectoderm) or gut (endoderm).

In the early mouse embryo[79, 80] L-CAM (uvomorulin, E-cadherin) is on both embryonic and extraembryonic epithelia. It is on all blastocysts at the lateral border of the trophectoderm but is absent from cell surfaces that contact the zona pellucida. In vitro it is expressed strongly on the inner cell mass and is gradually lost from trophoblastic cells. It is not expressed on the yolk sac or giant trophoblast cells. In vivo it is on embryonic and extraembryonic ectoderm, visceral endoderm chorion, the amniotic fold, and ectoplacental cone but is not seen on mesoderm or germ cells. P-Cadherin is found in the uterine decidua cells and later in the placenta. It is also expressed in the ectoplacental cone, and is has been suggested[79] that P-cadherin may link the embryo to the placenta. Its distribution in human placenta, however, is less clear. Human placenta has been examined only after 6 weeks' gestation,[81] but at this time it expressed higher levels of L-CAM than of P-cadherin.

Interactions between epithelia and mesenchymes that result in embryonic induction[82] lead to expression of genes for the morphoregulatory molecules in addition to those for tissue-specific and cell-specific molecules. Early epithelia express both N-CAM and L-CAM, but subsequently one or the other is lost in patterns that have allowed formulation of a set of place-dependent rules.[25] Other cell adhesion molecules such as N-cadherin and P-cadherin also follow place-dependent expression.[79, 83] In some tissues (e.g., the kidney or the feather) primary cell adhesion molecules can be up regulated and down regulated in cycles at different stages of histogenesis, and then secondary cell adhesion molecules appear at specific sites.

Examples of N-CAM expression[25] in different locations are shown in Fig. 3. In the developing eye (Fig. 3, *A*) it is seen in retina, cornea, and lens. When lens fibers form, N-CAM is not seen in lens but remains in

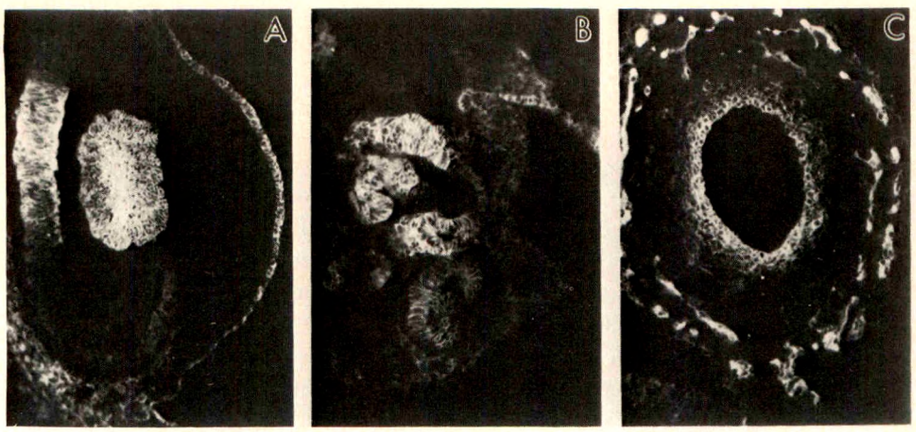


Fig. 3. Expression of N-CAM in tissues derived from all three germ layers.[25] **A**, N-CAM is expressed in retina, lens, and cornea of chicken eye at embryonic day 3; **B**, N-CAM in distal tubules of kidney at embryonic day 5; **C**, N-CAM on submucosa and ganglionic precursors in intestines at embryonic day 7. In most of these tissues N-CAM expression is markedly decreased as tissues mature, but N-CAM can be reexpressed in some tumors from these tissues, such as Wilms' tumor of the kidney.[119]

the proliferating lens epithelium. In kidney (Fig. 3, *B*) a series of cell adhesion molecule cycles is observed: L-CAM is seen on wolffian ducts (the inductor tissue) and, after induction, N-CAM appears on mesonephric mesenchyme; after induction, L-CAM appears on collecting tubules. In the formation of the gut (Fig. 3, *C*), N-CAM, as well as L-CAM, is seen in epithelia and then N-CAM disappears.[15, 25]

The importance of cell adhesion molecules in development is also emphasized by perturbation experiments with antibodies to specific cell adhesion molecules. Antibodies to N-CAM[84] or N-cadherin,[83] for example, perturb cell layers in retinal explants, whereas antibodies to Ng-CAM disrupt neurite fasciculation.[85] A variety of other such experiments shows that, in general, disruption of cell adhesion molecule binding alters morphologic features. Conversely, disruption of morphologic features can affect cell adhesion molecule expression, as seen, for example, when nerve-muscle interactions are disrupted. Myoblasts and myotubes express N-CAM, but after innervation of the muscle, N-CAM is down regulated and is barely detectable on muscle fibers. When the sciatic nerve is crushed or cut, levels of N-CAM messenger ribonucleic acid[86] and protein[87, 88] are markedly increased. Moreover, N-CAM and Ng-CAM expression are up regulated in the dorsal root ganglion of the affected segment and are down regulated in the ventral horn on the affected side.[88] After nerve regeneration, the normal patterns are restored.

Obviously the timing and regulation of cell adhesion molecule expression are critical processes for morphogenesis and for the maintenance of adult tissues. A variety of specific factors are also known to influence their expression. For example, estradiol enhances L-CAM expression in granulosa cells,[89] progesterone increases the expression of a cadherin in *Xenopus* oocytes,[90] and steroid hormones affect C-CAM (cell CAM-105) expression in rat uterus.[91] Nerve growth factor stimulates increased production of Ng-CAM/L1/NILE in PC12 cells[85, 92, 93] and Schwann cells[94]; it also can affect N-CAM levels in PC12 cells.[95, 96]

Several other agents have been shown to affect N-CAM expression. N-CAM levels were up regulated by treatment of embryonal cell lines with retinoic acid[97] and were down regulated when a rat neuroblastoma cell line was transfected with N-*myc*.[98] N-CAM expression in 3T3 cells has also been induced by transforming growth factor β.[99] Thyroidectomy leads to increased expression of N-CAM on the muscle surface, and the increase is reversed by thyroxine treatment.[100]

Cellular transfection

A variety of new information about cell adhesion molecules and their ability to influence other cellular processes has been obtained by transfecting cells with complementary deoxyribonucleic acid (cDNA) encoding specific cell adhesion molecules. The results have also verified previous analyses of cell adhesion molecule binding. For example mouse L cells, which do not express any known cell adhesion molecule, have been transfected with cDNA for a number of different cell adhesion molecules.[20, 71, 101-103, 139] In each case the specific cell adhesion molecule was synthesized and expressed on the cell surface. The transfected cells, unlike normal L cells, formed aggregates, and antibodies to the trans-

fected cell adhesion molecule blocked this aggregation. L-CAM-expressing cells[139] did not bind to N-CAM-expressing cells or to untransfected cells, verifying that L-CAM, like N-CAM, mediates cell adhesion by a homophilic mechanism.

Most cell adhesion molecules are transmembrane proteins, and a variety of data indicate that they interact with cytoskeletal elements. Actin filaments, for example, collect near boundaries between cells aggregated by L-CAM[20] or N-cadherin,[102] and spectrin binds to the large cytoplasmic domain form of N-CAM.[104] L-CAM interacts with cytoplasmic proteins called catenins,[105] and in extracts of MDCK cells it purifies with fodrin-ankyrin complexes.[106] Transfection with cell adhesion molecule cDNA has emphasized the significance of these interactions. For example, aggregation by cells transfected with L-CAM cDNA is reduced by cytochalasin D, which disrupts microfilaments, whereas aggregation of cells transfected with N-CAM cDNA is mildly decreased by nocodazole, which disrupts microtubules.[103] In addition, removal of as few as 30 amino acids from the carboxyl terminus of L-CAM destroyed its interaction with actin; the transfected cells did not aggregate even though they expressed truncated L-CAM on the cell surface.[103, 105, 107, 108]

As an extension of this approach, L-CAM/N-CAM chimeric cDNA that encoded the extracellular region of L-CAM coupled to the intracellular region of N-CAM was used.[103] As expected, cells expressing the L-CAM/N-CAM chimeric protein aggregated with the specificity of L-CAM but the aggregation was inhibited by nocodazole, which affects N-CAM binding, but not by cytochalasin D, which affects L-CAM binding. Cell adhesion molecule binding is thus influenced and can be regulated by cytoplasmic elements.

Transfected cells also reveal interactions between cell adhesion molecule binding and other cellular processes, such as junction formation by cell junctional molecules. The mouse sarcoma S180 cell line is a fibroblast-like cell that expresses no known cell adhesion molecule, aggregates weakly, if at all, and forms extremely few gap junctions or adherens junctions. When this cell line was transfected with L-CAM[20] or N-cadherin[102] cDNA, the cells aggregated via the specific cell adhesion molecule. Moreover, at confluence the cells were changed to a polygonal shape, formed an epithelioid sheet, and expressed significant numbers of adherens junctions and communicating gap junctions.

An important aspect of development is the sorting out of discrete populations of cells. Transfected cells provide a simplified, controlled system for examining the role of cell adhesion molecules in some aspects of these processes. In addition, cell sorting requires cell movement and is thus more complex than simple cell adhesion, so cell sorting assays can reveal other subtleties in cell adhesion molecule function. For example, cells expressing different cell adhesion molecules can sort from each other,[101, 109, 110] but so can cells expressing different amounts of the same cell adhesion molecule.[109] Similarly, cells expressing the L-CAM/N-CAM chimera described above bind to L-CAM-expressing cells but sort out from them and do not sort from untransfected cells[103]; presumably the cytoplasmic anchoring of the chimeric molecule is too weak to withstand the mechanical forces of cell sorting. The sorting of cells expressing two cell adhesion molecules from cells expressing one is complex[109] but appears to be directed by the levels of expression of the shared cell adhesion molecule. Cell adhesion molecules thus are critical to cell sorting, but factors such as levels of expression, cytoplasmic interactions, and the combination on any one cell are as important to this process as specificity.

Cell adhesion molecules in disease

The roles of cell adhesion molecules in disease and their potential use as diagnostic indicators are just beginning to be assessed, but a variety of conditions have already been found that involve changes in cell adhesion molecule expression. Alterations of N-CAM levels have been seen in degenerative disease, developmental defects, and toxic conditions.[13] For example, N-CAM levels were increased in the amniotic fluid of mothers carrying fetuses with neural tube defects,[111] and in one study N-CAM increased in the cerebrospinal fluid of patients with multiple sclerosis in parallel with their clinical improvement.[112] Cell adhesion molecule expression patterns are changed in a variety of muscular disorders, and resemble those seen in regenerating muscles.[113-115]

Both up and down regulation of cell adhesion molecules has been observed in tumors and transformed cells. N-CAM is down regulated in neural retinal cells transformed with Rous sarcoma virus,[116] and L-CAM (uvomorulin) is down regulated in MDCK cells transformed with Harvey or Moloney sarcoma viruses.[117] Moreover, the ability of the MDCK cells to invade collagen gels or heart explants increased with decreased L-CAM expression. Expression of L-CAM also has been shown to be inversely correlated with metastatic potential in sublines of the murine ovarian tumor line OV2944.[118] In contrast to these down regulations, N-CAM expression is increased in Wilms' tumor,[119] small cell lung carcinomas,[120-122] Ewing's sarcoma,[123] and human neuroblastoma,[120, 123, 124] and in most cases the embryonic form of N-CAM is expressed. In lung carcinomas[81] both L-CAM and P-cadherin are present in amounts and locations that parallel their expression in normal tissues, and they are detected at similar levels in lymph node metastases of these tumors.

Several molecules associated with tumor formation are members of the N-CAM family. These include the carcinoembyonic antigen,[125-128] the biliary glycoprotein BGP1,[129] melanoma antigen MUC18,[130] and a gene deleted in colon carcinoma (DCC).[131] Carcinoembyonic antigen is present in embryonic and transformed colonic epithelia and has been shown recently to be a cell adhesion molecule.[33] MUC18 is found on normal vascular smooth muscle[132] and on the surface of melanoma cells, where its appearance correlates with metastatic potential and poor prognosis. The DCC gene product has been postulated to be a tumor suppressor.[131, 133]

Overall the study of cell adhesion molecules in pathologic tissues promises to lead to a variety of new diagnostic reagents. It should also provide new insights into aspects of cell adhesion molecule function.

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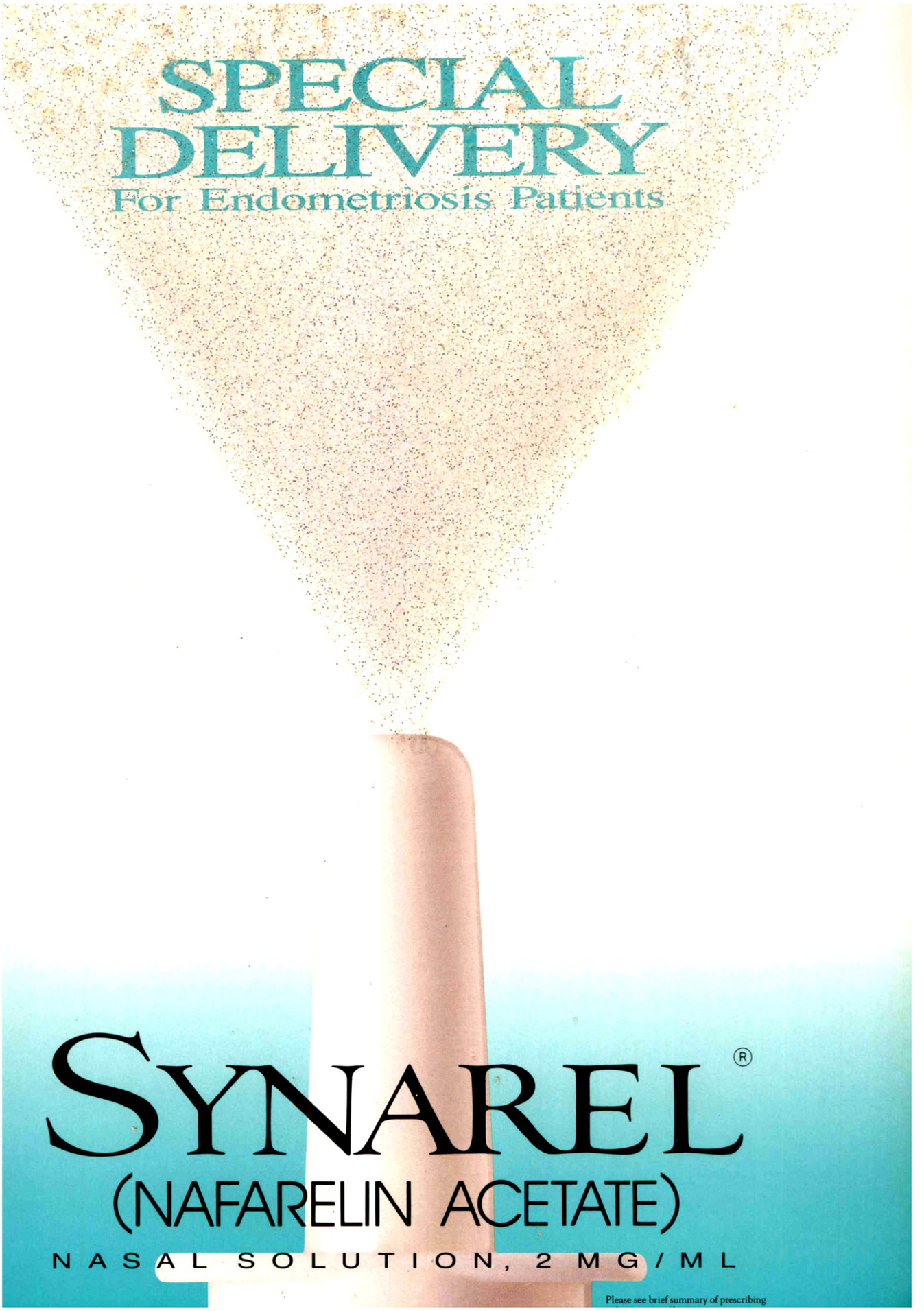
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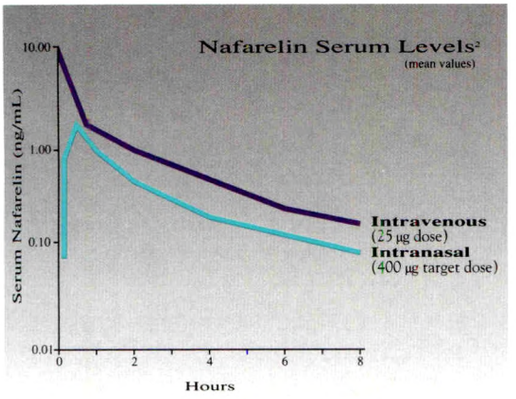
Of all the available treatment options for endometriosis, GnRH agonists are emerging as a preferred therapeutic option—and SYNAREL® (nafarelin acetate) is setting the pace.

One-of-a-kind intranasal administration

Unlike other GnRH agonists, SYNAREL is 200 times as potent as native GnRH—which is why SYNAREL can be administered in a highly accurate, controlled-dose intranasal form. Intranasal delivery is preferred by physicians[1†] for patient convenience, as well as for ease of patient management should allergic or adverse events occur.

Proven absorption for reliable control

In pharmacokinetic studies, SYNAREL was rapidly absorbed into systemic circulation following intranasal administration, with maximum serum concentrations achieved between

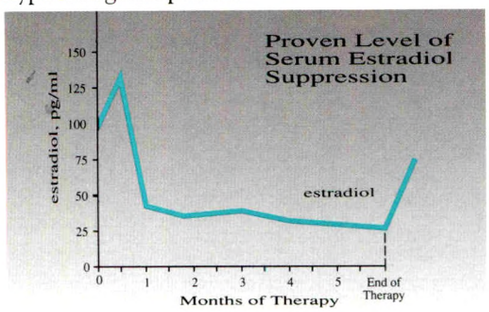


Intranasal administration produces nafarelin serum levels comparable to IV. dosing[2]

10 and 40 minutes. And, SYNAREL therapy was well tolerated, with a low incidence of nasal irritation (10%).

Outstanding resolution of symptoms

B.I.D. controlled-dose intranasal administration provides serum estradiol levels that result in outstanding clinical efficacy in the management of endometriosis, while producing minimal impact on bone and a predictable hypoestrogenic profile.[‡]



Serum estradiol concentration during treatment with SYNAREL 200 µg B.I.D. (n = 73)

In clinical studies, 94% of patients taking SYNAREL reported improvement of symptoms. Furthermore, 86% of patients taking SYNAREL showed improvement of endometriotic lesions (as measured by AFS scores) after the 6-month course of therapy.

Lowers estrogen without adding androgen

SYNAREL exhibits fewer masculinizing side effects than danazol. (The most frequent side effects are those of hypoestrogenism.)

The GnRH analog more and more physicians prescribe

Today, more and more physicians are prescribing SYNAREL for the management of endometriosis[3]—and more and more patients are experiencing the important clinical benefits intranasal SYNAREL has been proven to provide.



SYNAREL®

(NAFARELIN ACETATE)

NASAL SOLUTION, 2 MG/ML

Setting The Pace In Endometriosis Therapy

1. Syntex document #829-243-A.
2. Syntex document #829-243-B.
3. Syntex document #829-243-C.

*Gonadotropin-releasing hormone analog.

†In a written survey conducted at the 38th Annual Meeting of the American College of Obstetricians and Gynecologists (May 1990), 299 physicians who prescribe GnRH analogs for the management of endometriosis were asked whether they preferred the intranasal or depot injection route of drug administration, and why.

‡ There is a small, largely reversible loss in bone density with 6-month treatment. Use of Synarel for longer than the recommended 6 months or in the presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Please see brief summary of prescribing information on last page.

The Only GnRH* Agonist With The Dosing Distinction Of Intranasal Delivery

B.I.D. controlled-dose intranasal administration offers important clinical benefits, including highly accurate metered-dose delivery and proven absorption through nasal mucosa. Pregnancy should be ruled out before therapy with SYNAREL is initiated, and patients should be advised to use a nonhormonal method of contraception when taking SYNAREL.

Intranasal administration provides serum estradiol levels that result in outstanding clinical efficacy in the management of endometriosis, while producing minimal impact on bone and a predictable hypoestrogenic profile.† Retreatment cannot be recommended since safety data for retreatment are not currently available.

Physician-preferred GnRH agonist delivery system for patient convenience.

Intranasal administration facilitates ease of patient management should allergic or adverse events occur.

For more information, please call: 1-800-727-0400



*Gonadotropin-releasing hormone analog.

† There is a small, largely reversible loss in bone density with 6-month treatment. Use of Synarel for longer than the recommended 6 months or in the presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

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829-H2-243-90



SYNAREL®
(NAFARELIN ACETATE)
NASAL SOLUTION, 2 MG/ML

Setting The Pace In Endometriosis Therapy

BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloride, glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.

INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.

CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.

WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.

When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.

PRECAUTIONS Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. Patients missing successive doses may experience breakthrough bleeding.

2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients.

3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician.

4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients.

5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content.

6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption.

7. Retreatment cannot be recommended since safety data beyond 6 months are not available.

Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.

Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.

Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.

Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (± SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

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Periovulatory calcium channel blockade enhances reproductive performance in an animal model for endometriosis-associated subfertility

Alex Steinleitner, MD, Hovey Lambert, PhD, Mariluz Suarez, BS, Nurys Serpa, BS, Beverly Robin, BS, and Bernard Cantor, MD

Miami Beach, Florida

Current evidence suggests that peritoneal inflammatory cell hyperactivation may be the essential pathologic abnormality in patients with endometriosis-associated subfertility. In these experiments we utilized an animal model to evaluate the use of an immunomodulatory agent as an alternative treatment for endometriosis-associated subfertility. The right uterine horn of 12 golden hamsters was resected and 2 × 2 mm squares were explanted onto the left uterine mesentery. Controls included hamsters undergoing uterine horn resection without endometrial explantation ($n = 6$) and nonsurgically treated animals ($n = 6$). Ovulation induction was initiated 6 weeks postoperatively with 40 IU of pregnant mare's serum gonadotropin on day 1 and 40 IU of human chorionic gonadotropin on day 3. Groups of six explant-bearing hamsters and six nonsurgical control hamsters were treated with verapamil (250 μg/kg every 8 hours subcutaneously), a calcium channel–blocking agent known to inhibit macrophage activation, from day 1 to 48 hours after administration of human chorionic gonadotropin. Animals were then killed and the number of embryos and oocytes in left tube counted as a reflection of reproductive performance. Fertilization was completely inhibited in animals bearing uterine explants. Treatment with verapamil dramatically reversed this effect. These data suggest that periovulatory treatment with an immunomodulatory agent such as verapamil may be an effective alternative to conventional treatment for endometriosis-associated subfertility. (Am J Obstet Gynecol 1991;164:949-52.)

Key words: Endometriosis, calcium channel blockade, verapamil, infertility

Contemporary thought in reproductive endocrinology holds that aberrations in intraperitoneal nonspecific immunity may be the essential pathologic entity contributing to subfertility in patients with minimal or mild endometriosis.[1] This hypothesis is supported by the finding of increased numbers of activated peritoneal inflammatory cells[2] and elevated levels of gametotoxic inflammatory mediators[3, 4] in the peritoneal fluid of patients with endometriosis when compared with fertile controls. However, despite increasing acceptance of these findings as the etiologic basis for endometriosis-associated subfertility, therapy for these patients continues to be based on the medical or surgical eradication of endometriotic implants.

In this article we describe experiments that explore an alternative approach to the treatment of impaired early reproductive performance in endometriosis, using the calcium channel–blocking drug verapamil as an immunomodulatory agent to reduce intraperitoneal inflammatory cell activation in an animal model for endometriosis-associated subfertility.

From the Department of Obstetrics and Gynecology, Mount Sinai Medical Center.
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Reprint requests: Alex Steinleitner, MD, Department of Obstetrics and Gynecology, Mount Sinai Medical Center, 4300 Alton Road, Gumenick Building, 2nd Floor, Miami Beach, FL 33140.
6/6/27337

Material and methods

Experimental procedures used in this study were based on rodent models for endometriosis-associated subfertility described by Golan[5] and Kaplan et al.[6] All animals were maintained on a constant 14-hour-light/10-hour-dark cycle, fed standard laboratory chow, and given water as desired for 7 to 10 days before use in these studies. Animal care procedures were reviewed by the Animal Reseach and Medical Research Committees of Mount Sinai Medical Center (Miami Beach) and were performed in accordance with the standards set in the *NIH Guide for Care and Use of Laboratory Animals.*

Sexually mature female golden hamsters (6 weeks old, 150 gm, Harlan Sprague-Dawley, Indianapolis) were used in a model for surgically induced pelvic endometriosis. Midline laparotomies were performed with animals under pentobarbital anesthesia; prophy-

lactic antibiotics (0.1 mg of penicillin G) were given and a clean, but not sterile, technique was used. At laparotomy the right uterine horn was excised, and care was taken to preserve the right ovary. The excised uterine horn was opened longitudinally and cut into four 2 × 2 mm specimens. In 12 animals an endometriosis-like lesion was induced by suturing the excised uterine specimens onto the left uterine mesentery with 6-0 nylon sutures. An additional six animals served as surgical controls, undergoing resection of the right uterine horn without subsequent explantation. In each instance incisions in the hamsters were closed in two layers with 4-0 polyglycolic acid sutures, and the animals were returned to the vivarium for observation.

Six weeks after operation all hamsters were subjected to ovulation induction with 40 IU of pregnant mare's serum gonadotropin on cycle day 1, followed by 40 IU of human chorionic gonadotropin on day 3. Hamsters bearing uterine explants were randomly divided into two groups for treatment with subcutaneous injections of either verapamil (250 μg/kg) or vehicle (phosphate-buffered saline solution), given every 8 hours starting with the initiation of ovulation induction. Concurrently, ovulation induction was begun in 12 animals that had not undergone surgery; groups of six animals were treated with either verapamil or phosphate-buffered saline solution injections, as described above.

Immediately after treatment with human chorionic gonadotropin hamsters were placed in individual mating chambers for 48 hours with male animals of established fertility. Subsequently, all animals were killed by cervical dislocation for collection of the left uterine horn. Injections of study medications (verapamil, vehicle) were continued until the time of death. Unfertilized oocytes and embryos were flushed from the left uterine horn and counted as a reflection of reproductive performance.

Nonparametric statistics (Kruskal-Wallis, Mann-Whitney U tests) were used to compare the number of oocytes and embryos and the rates of fertilization in the various experimental groups. All data are reported as the mean ± SEM.

Results

Surgical procedures, ovarian hyperstimulation, and verapamil treatment were well tolerated by all animals. The entire complement of hamsters was available for analysis in this experiment.

Number of gametes flushed from the left uterine horn of hamsters treated with simple excision of the right uterine horn (17.2 ± 0.9) was not different from that of saline solution–treated (16 ± 1.5) or verapamil-treated (18.8 ± 1.4) nonsurgical controls. Fertilization rates were 100% in these groups. In contrast, autologous explantation of resected uterine tissue to the left uterine mesentery completely inhibited fertilization (0%). Ovulation was not affected in these hamsters (17.3 ± 1.5 nonfertilized oocytes per animal). Periovulatory treatment with verapamil entirely reversed the effect of surgically induced endometriosis on fertilization. Number of embryos (19.5 ± 1.8) and fertilization rate (100%) in these animals were not different from those of surgical and nonsurgical controls.

Comment

Current evidence suggests that subfertility in patients with minimal or mild endometriosis results from an adverse effect of activated immunocompetent cells on early reproductive events. Data supporting this hypothesis include the finding of increased numbers of activated peritoneal macrophages and lymphocytes in patients with endometriosis.[1, 2] Activated peritoneal macrophages have been found to specifically inhibit fertilization in animal models.[7] Peritoneal fluid from patients with endometriosis has been shown to contain elevated levels of gametotoxic inflammatory mediators (e.g., interleukin-1,[4] tumor necrosis factor[3]) and to inhibit gamete interaction and embryo development in both in vitro[8, 9] and in vivo models.[10] Recent evidence suggests that the aberrant peritoneal inflammatory environment may be a consequence of the production of complement factor 3 (a potent inflammatory mediator) by endometriotic implants.[11]

In spite of this evolving understanding of the pathophysiologic profile of endometriosis-associated subfertility, current approaches to the treatment of endometriosis-associated subfertility remain empiric and are based on the eradication of visible endometriotic implants. In many instances these therapies have proved unsatisfactory to both patient and practitioner. Medical suppression of endometriotic implants entails the use of medications that impart undesirable side effects (e.g., danazol, gonadotropin-releasing hormone agonists). Patients must forgo attempting conception for the duration of the suppression cycle. Subsequent recurrence of endometriosis is common.[12] It is important that neither medical nor surgical eradication of stage I or stage II endometriosis has been shown to be superior to expectant management in controlled trials.[13-16] The need to develop effective treatment modalities not limited by these restrictions is evident.

Studies in our laboratory have focused on the use of immunomodulatory agents as an alternative means of treating endometriosis-associated subfertility. In this study we evaluated the effect of calcium channel blockade on the inhibition of early reproductive performance associated with uterine explantation in the hamster model for endometriosis. Although calcium channel–blocking agents are not classic "antiinflammatory drugs," drugs of this class are known to exert

diverse effects on leukocyte function. Macrophage activation is inhibited by verapamil and nifedipine in vitro.[17] Macrophage cytolytic factor[18] and concanavalin A–stimulated superoxide ion[19] production is reduced by verapamil and its analogs. Production of interferon gamma is diminished by most calcium antagonists.[20] Lymphocyte migration induced by interleukin-1 and interleukin-8 is markedly inhibited by calcium blockade in vitro.[21] T-cell proliferation,[22] activation,[23] and capping[24] are impaired by a variety of calcium channel blockers. Natural killer activity is reduced in the presence of both verapamil and nifedipine.[25]

The data obtained in this study demonstrate calcium blockade to be an effective means of abrogating the adverse influence of uterine explants on early reproductive performance in this model. Periovulatory verapamil administration resulted in a complete reversal of the inhibition of fertilization observed in vehicle-treated hamsters. Ovulation was not affected in these animals. It is significant that this activity is not restricted to drugs with calcium channel–blocking properties. We have obtained similar results after periovulatory administration of the methylxanthine analog pentoxifylline in the murine syngeneic macrophage transfer model for endometriosis–peritoneal fluid environment[26] and in a hamster model identical to that used in this study.[27] We hypothesize that this phenomenon is the result of immunomodulation of intraperitoneal inflammatory activity, resulting in a consequent inhibition of monokine and lymphokine production or cell-mediated immune events responsible for impaired reproductive performance.

Although these early data are provocative, it is premature to suggest that these agents be used in clinical practice. Drugs used as immunomodulators in reproductive medicine must meet stringent criteria. Immunomodulatory agents must reduce inflammatory cell activity without adversely affecting ovulation, tubal motility, or nidation. Medications must be known to have minimal teratogenic potential. Ideally, agents should be free from noxious side effects. Until an appropriate agent has been thoroughly evaluated for safety and efficacy in a controlled clinical trial, we would recommend restraint in the application of these drugs for the routine treatment of patients with endometriosis.

In summary, these preliminary animal data provide a paradigm for an alternative approach to the treatment of endometriosis-associated subfertility, which is directed to the essential pathophysiologic parameters of this condition. Treatment with immunomodulatory agents holds the potential for supplying a safe and effective means of enhancing fertility without subjecting patients to the noxious side effects of contemporary treatment modalities. Further study of appropriate immunomodulators in the treatment of endometriosis-associated subfertility is indicated.

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Discussion

Dr. Roland A. Pattillo, Milwaukee, Wisconsin. Your observation of verapamil-induced enhanced fertility is indeed of interest, and I commend you on this exciting finding and article.

Did you measure natural killer cell activity and was it altered as you indicated that decrease in natural killer cell activity may indeed be associated with the immunomodulatory effects that were observed?

Dr. Steinleitner (Closing). At this point we have not measured natural killer cell activity or the activity of other immunocompetent cells in our model, because of the limitations of our present laboratory.

I think it's important to note that in 1990 we don't know what the principal effector cell is. However, if we use an immunomodulator that has broad specificity, then it is possible to treat this in an, if you will, empiric fashion, going after all possible effectors of this condition.

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Macrophage-conditioned media enhance endometrial stromal cell proliferation in vitro

David L. Olive, MD, Iris Montoya, BS, Robert M. Riehl, PhD, and Robert S. Schenken, MD

San Antonio, Texas

It has been hypothesized that the activated peritoneal macrophages of endometriosis, rather than being a response to ectopic endometrium, may contribute to its pathogenesis. This study attempts to define the effect of macrophage-derived growth factors on endometrial stromal cell growth in vitro, as well as the interaction between estrogen and these growth factors. Mouse endometrial stromal cells were prepared and cultured in either a serum-containing, insulin-free medium or a serum-free, insulin-containing medium, and the effects of adding 10% macrophage-conditioned medium on [^{3}H]thymidine incorporation were assessed. Results indicate that macrophage-conditioned medium will increase incorporation in the presence of insulin but not in insulin-free media. Serum can substitute for macrophage-conditioned medium, but the two together show no additive or synergistic effect. The effect of estrogen on this system was determined, and results indicate that estrogen will perform the function of insulin, with an optimal concentration of 10^{-10} to 10^{-12} mol/L. Thus macrophage-conditioned medium appears to function as a competence growth factor, and estrogen (in appropriate concentrations) functions as a progression growth factor. (Am J Obstet Gynecol 1991;164:953-8.)

Key words: Macrophage, endometriosis, estrogen, stromal cell

Endometriosis, a gynecologic disorder involving ectopic growth of endometrial tissue, has long been tied to immunologic abnormalities. Several investigators have identified an abnormality of peritoneal macrophages in such women, demonstrating an increase in macrophage number[1, 2] and activation level[2] in association with the disease. Additional studies[2, 3] have reported excessive production of macrophage secretory products in the presence of endometriosis.

A prevalent hypothesis has been that the ectopic endometrium incites an intraperitoneal inflammatory response, resulting in recruitment and differentiation of peritoneal macrophages. However, a recent longitudinal study[4] in the rabbit calls this theory into question by failing to demonstrate a change in macrophage number or function after induction of experimental endometriosis. Thus, whereas an association between endometriosis and abnormal macrophage function exists, no direct cause-and-effect relationship has been established.

An alternative hypothesis is the converse: A primary disorder of macrophage number or function may contribute directly to ectopic endometrial growth. Macrophage-derived growth factors that enhance the growth of fibroblast cell lines in vitro have been identified.[5, 6] In addition, women with endometriosis have been shown to possess peritoneal macrophages with excessive secretion of such growth factors.[7]

In this study we propose to define the effect of macrophage-derived growth factors on endometrial stromal cell growth in vitro. We have further sought to characterize the exact role of such factors in the cell growth process. Our results suggest that macrophage-derived growth factors function as competence factors, allowing endometrial stroma to respond to mitogenic influences by increasing their rate of proliferation.

From the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio.
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Reprint requests: David L. Olive, MD, 7703 Floyd Curl, San Antonio, TX 78284-7836.
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Material and methods

Animals and cell lines. Three- to five-month-old C57BL/6J mice and 8- to 12-month-old C3H/Hej mice (Jackson Laboratory, Bar Harbor, Me.) were used for harvesting peritoneal macrophages. Uterine tissue was obtained only from the C57BL/6J animals. The P388D$_1$ murine cell line (American Type Culture Collection, Rockville, Md.) was used for production of macrophage-conditioned media.

Chemicals. Nutridoma-SP media and insulin were purchased from Boehringher-Mannheim Biochemicals (Indianapolis). L-Glutamine, sodium pyruvate, gentamicin, and RPMI 1640 powdered media with phenol red were purchased from Hazelton Biologics, Inc. (Le-

nexa, Kan.). HEPES buffer and RPMI 1640 powdered media, phenol red free, were purchased from Irvine Scientific (Santa Ana, Calif.). Sodium chloride, potassium chloride, sodium bicarbonate, potassium orthophosphate, calcium chloride monohydrate, sodium lactate, glucose, bovine serum albumin, activated Norit-A charcoal, β-estradiol, deoxyribonuclease I, and phenol red solution were purchased from Sigma Chemical Corporation (St. Louis). Magnesium sulfate heptahydrate and sodium pyruvate were purchased from Fisher Scientific (Fair Lawn, N.J.). Dextran T-70 was purchased from Pharmacia LKB Biotechnology (Piscataway, N.J.), and defined fetal calf serum was obtained from Hyclone Laboratories (Logan, Ut.). Collagenase was purchased from Cooper Biochemical Corporation (Malvern, Pa.). [^{3}H]Thymidine was obtained from ICN Radiochemicals (Irvine, Calif.). Peptone was purchased from Difco Laboratories (Detroit).

Media preparation. The media used for isolation of endometrial stromal cells were modified Brinster's medium,[8] pH 7.2, consisting of 115.48 mmol/L sodium chloride, 4.78 mmol/L potassium chloride, 1.19 mmol/L, 25 mmol/L sodium bicarbonate, 1.19 mmol/L magnesium sulfate, 20 mmol/L heptahydrate lactic acid, 0.5 mmol/L sodium pyruvate, 5.5 mmol/L glucose, and 5 gm/L bovine serum albumin.

For cell culture and proliferation assays, Nutridoma-SP (a serum-free medium containing insulin) was prepared with phenol red–depleted RPMI 1640, 1 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 10 to 25 mmol/L HEPES buffer, 25 μg/ml gentamicin, and 0.1% Nutridoma-SP base.

RPMI 1640 media for cell culture and proliferation assays consisted of 1 package of RPMI 1640 powder per liter of Millipore-filtered water, plus 2 gm sodium bicarbonate, pH adjusted to 7.3. When used for culture, 10% fetal bovine serum was added to produce a serum-containing medium without insulin.

Serum samples were stripped of sex steroids by addition of 10 mg/ml Dextran-T70 and 100 mg/ml Norit-A charcoal overnight at 4° C. The next morning the serum was filtered through 0.45, 0.2, and 0.1 μm filters into sterile containers and stored at 4° C.

Isolation of endometrial stromal cells. Endometrial stroma were isolated by a modification of the method of Riehl et al.[9] Briefly, the mice were put to death by cervical dislocation, and uteri were excised and placed immediately into modified Brinster's medium at 24° C. Fat and connective tissue were removed and the uteri were separated into individual horns. Each uterine horn was everted and placed into a 125 ml Erlenmeyer flask containing modified Brinster's medium with 1% (wt/vol) bovine serum albumin previously added. Each flask was gently agitated to remove dead cells and debris from the mucosal surface. Each horn was then removed with forceps and placed into a 50 ml test tube containing modified Brinster's medium, plus 1 mg/ml collagenase, 0.05% (wt/vol) deoxyribonuclease, and 0.5 mmol/L calcium chloride. The tubes were secured, immersed in a water bath at 37° C, and agitated for 30 minutes. At the end of the digestion period, each horn was drawn 25 times into the plastic tip of a 5000-P Pipetman (Rainin Instrument Co., Emeryville, Calif.) that had been cut to increase the bore diameter of the tip just large enough to accept the uterine horn. The solution was then filtered to remove epithelial cells. The uterine horn was then placed in 5 ml of fresh enzymatic solution, secured in a water bath, and agitated for 30 minutes. The horns were then drawn through the pipette tip 25 times, and the tube was again agitated for 30 minutes. The solution was then filtered and stromal cells were recovered. These cells were then washed twice in modified Brinster's media plus 1% bovine serum albumin.

Preparation of macrophage-conditioned media. Resident peritoneal macrophages were collected from C57BL/6J mice after cervical dislocation and rapid entry into the peritoneal cavity, where free fluid was collected and the cavity was washed with RPMI 1640. Peptone-stimulated peritoneal macrophages were induced by intraperitoneal injection of 1.0 ml 10% peptone into C57BL/6J and C3H/Hej mice. Three days later the animals were killed and the cells were collected as previously described.

All macrophages and the P388D$_1$ murine macrophage-like cell line were plated at 10^5 cells/ml in Nutridoma medium and incubated 72 hours. Culture supernatants were then collected, passed through a 0.4 μm filter, and stored for later use at −20° C.

Proliferation assay. Endometrial stromal cells, once isolated, were added to 96-well microliter plates (Corning Industries, Corning, N.Y.) at concentrations of 5×10^3 to 10^5 cells per well. In most cases the cells were immediately treated with an experimental medium, but in some experiments they were cultured for 24 hours in Nutridoma medium before addition of experimental media. Forty hours after exposure to an experimental medium, [^{3}H]thymidine was added and the cells were cultured an additional 8 hours. The wells were then washed in a Mini-Mesh II irrigation-aspiration system (MA Bioproducts, Walkersville, Md.) and the cells were filtered. Incorporated [^{3}H]thymidine was counted with a Tri-Carb 460C liquid scintillation system (Packard Instrument Company, Meridan, Conn.). All assays were performed in triplicate or quadruplicate.

Statistical analysis. All calculations were performed with the True Epistat program (Epistat Services, Round Rock, Tex.) on an IBM 386 personal computer (IBM Corporation, Boca Raton, Fla.). Two-sample comparisons were performed with Student's t test for inde-

pendent samples. One-way analysis of variance was used for comparison of more than two independent samples. Homogeneity of variance was assessed with Bartlett's test. Multiple comparisons were performed with the Newman-Keuls multiple range test.

Results

Cell density. Stromal cells were added at densities of 5×10^3 to 10^5 per well and cultured in serum-containing media. A linear relationship was noted between densities of 10^4 to 10^5 cells per well and radioisotope incorporation, with the least variance noted at 5×10^4 cells per well (data not shown). This density was thus chosen for the majority of experiments.

Effect of insulin. As insulin is a known progression factor for fibroblasts in supraphysiologic doses, an attempt was made to determine the dose response of endometrial stromal cells to insulin in a competence factor–containing medium. Cells were plated at 5×10^4 per well in RPMI 1640 with 10% fetal bovine serum. The addition of 10 ng/ml to 1 μg/ml insulin resulted in significant increases in thymidine incorporation ($p = 0.0005$), as seen in Fig. 1.

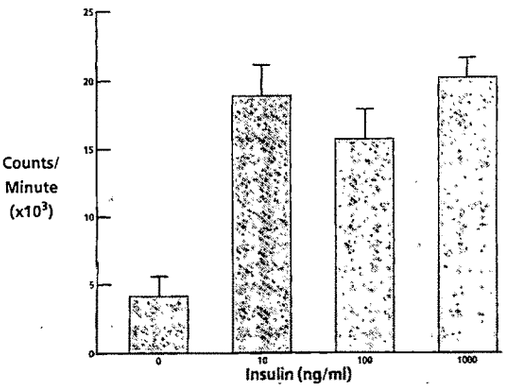


Fig. 1. Effect of addition of insulin (10, 100, and 1000 ng/ml) to serum-containing media on stromal cell proliferation as measured by [³H]thymidine incorporation (mean ± SEM in counts per minute). Cells were harvested after 48 hours of plating.

Effect of macrophage-conditioned medium. Stromal cultures of 10^4 cells per well were treated with serum-free media with or without 10% macrophage-conditioned medium from a variety of sources (Fig. 2). An increase in thymidine incorporation was noted in each of the macrophage-conditioned medium–treated samples, and all differed significantly from cells not exposed to macrophage-conditioned medium ($p = 0.0076$).

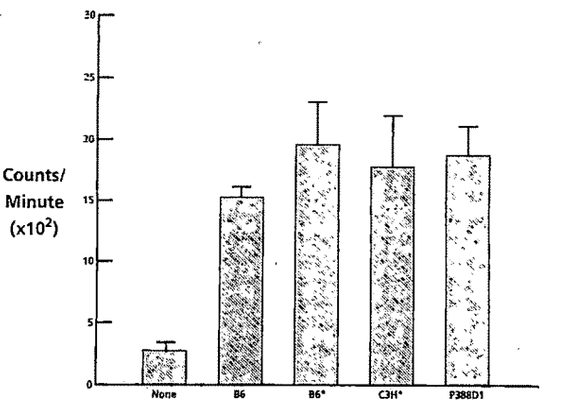


Fig. 2. Effect of addition of 10% macrophage-conditioned media on stromal cell proliferation as measured by [³H]thymidine incorporation (mean ± SEM in counts per minute). Cells were harvested 48 hours after plating. *None*, No macrophage-conditioned medium supplementation; *B6*, macrophage-conditioned medium from resident macrophages of C57BL/6J mice; *B6**, macrophage-conditioned medium from peptone-stimulated macrophages of C57BL/6J mice; *C3H**, macrophage-conditioned medium from peptone-stimulated macrophages of C3H/Hej mice; *P388D$_1$*, macrophage-conditioned medium from P388D$_1$ murine cell line.

To determine the precise effect of macrophage-conditioned medium, radioisotope incorporation was assessed in the presence of two culture media: one containing a known competence factor but lacking a progression factor (RPMI 1640 plus 10% fetal bovine serum) and one containing a known progression factor but lacking a competence factor (Nutridoma). Cells were then cultured at 5×10^4 per well. The results can be seen in Fig. 3. In the presence of serum-containing media without insulin, macrophage-conditioned medium failed to significantly affect proliferation. However, with the serum-free media with insulin, macrophage-conditioned medium significantly increased cell proliferation ($p = 0.00007$). Thus macrophage-conditioned medium is acting as a competence factor to promote endometrial cell growth. Finally, in a serum-free medium with insulin added, the presence or absence of macrophage-conditioned medium has no effect as thymidine incorporation is stimulated in either case.

Timing of addition of macrophage-conditioned medium. To assess whether the effect of macrophage-conditioned medium as a competence factor was acting to enhance initial cell survival or attachment or whether it was truly functioning to condition cells to respond to progression factor, addition of macrophage-conditioned medium was performed 24 hours after initial cell plating. Again the addition of macrophage-conditioned medium to the serum-free medium with insulin resulted in a significant increase in [³H]thymidine incorporation ($p = 0.02$) (Fig. 4).

Effect of estrogen. To assess the role of estrogen in endometrial stromal proliferation, cells were cultured

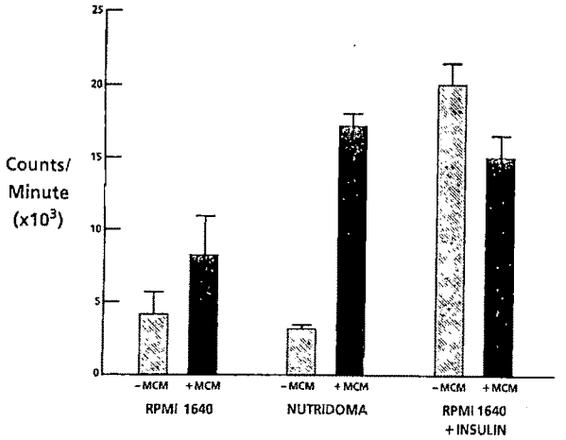


Fig. 3. Effect of addition of 10% macrophage-conditioned media on stromal cell proliferation as measured by [^{3}H]thymidine incorporation (mean ± SEM in counts per minute). *RPMI 1640*, Media containing competence factor with no progression factor; *Nutridoma*, media containing progression factor with no competence factor; *RPMI 1640 + insulin*, media containing competence factor with progression factor added (insulin, 1 μg/ml); *−MCM*, no macrophage-conditioned medium supplementation; *+MCM*, supplementation of culture medium with 10% macrophage-conditioned medium.

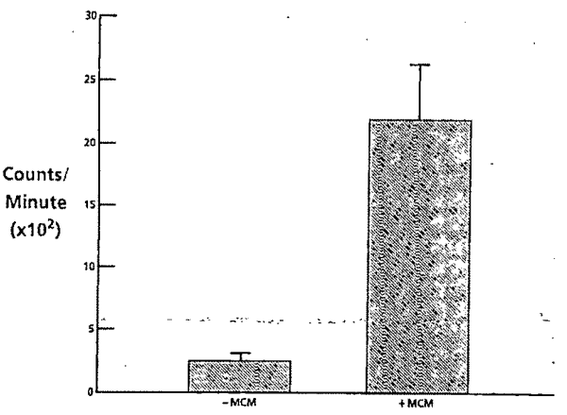


Fig. 4. Effect of addition of 10% macrophage-conditioned media on stromal cell proliferation as measured by [^{3}H]thymidine incorporation (mean ± SEM in counts per minute). Cells were cultured in Nutridoma (serum-free, insulin-containing) media for 24 hours before supplementation. Cells were harvested 72 hours after plating.

at 5×10^4 per well in insulin-free, serum-containing media with increasing concentrations of estradiol (Fig. 5). The addition of estrogen clearly increased thymidine incorporation ($p = 0.0009$), with maximal effect seen at 10^{-10} to 10^{-12} mol/L. Thus estradiol seems to complement serum (and act as a progression factor) in affecting stromal cell growth.

Conversely, when cells were cultured in a serum-free medium with insulin, estrogen had no affect on proliferation (Fig. 6). When 10% macrophage-conditioned

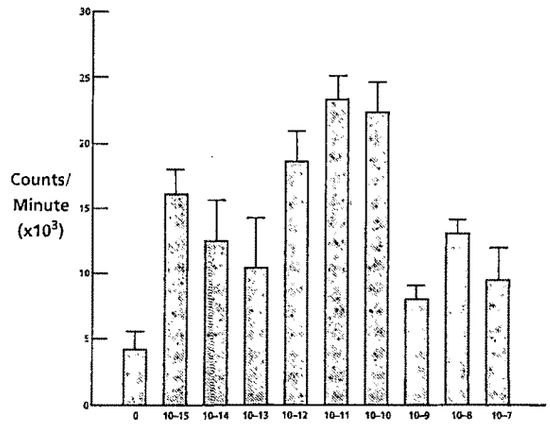


Fig. 5. Effect of addition of estradiol on stromal cell proliferation as measured by [^{3}H]thymidine incorporation (mean ± SEM in counts per minute). Cells were cultured in RPMI 1640 with 10% fetal bovine serum. Cells were harvested after 48 hours of plating.

medium was added to the serum-free medium with insulin, increased proliferation was noted for all samples, and estradiol failed to increase proliferation above that of the non-estradiol-treated control (Fig. 7). Thus estradiol performs the same function as insulin in enhancing stromal cell growth and demonstrates no additive or synergistic response.

Comment

The association between endometriosis and peritoneal macrophages has been recognized since Haney et al.[1] first reported an increase in macrophages in infertile women with the disorder. Subsequent studies have confirmed these findings and have demonstrated an increased level of activation[2] and increased membrane capping.[10] However, this association has not gone uncontested. Olive et al.[11] found an increase in peritoneal macrophage number to be associated with otherwise unexplained infertility, with no relationship between endometriosis and cell number. Hill et al.[12] also stated that the increase in macrophage number seen in infertile women with endometriosis was also found in women with unexplained infertility. Thus the presence of activated macrophages in increased number in endometriosis patients may be a function more of their infertility than of their implants.

Nevertheless, the hypothesis persists that ectopic endometrium incites an intraperitoneal inflammatory response, resulting in recruitment and differentiation of peritoneal macrophages. Indeed, peritoneal fluid lysozyme levels confirm a localized intraperitoneal inflammation in these women,[3] but a cause-and-effect relationship between implants and inflammatory cells has never been demonstrated. Recently a longitudinal

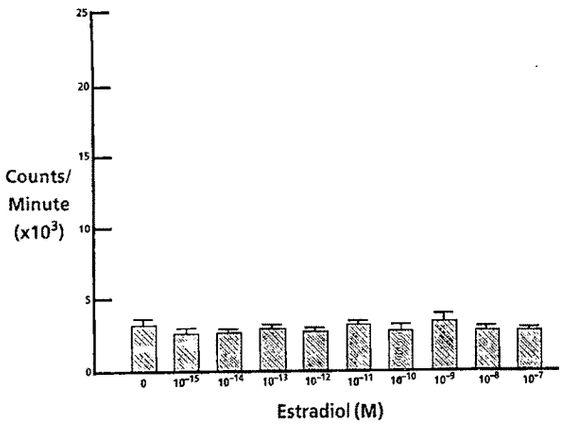


Fig. 6. Effect of addition of estradiol on stromal cell proliferation as measured by [³H]thymidine incorporation (mean ± SEM in counts per minute). Cells were cultured in Nutridoma (serum-free, insulin-containing) media. Cells were harvested after 48 hours of plating.

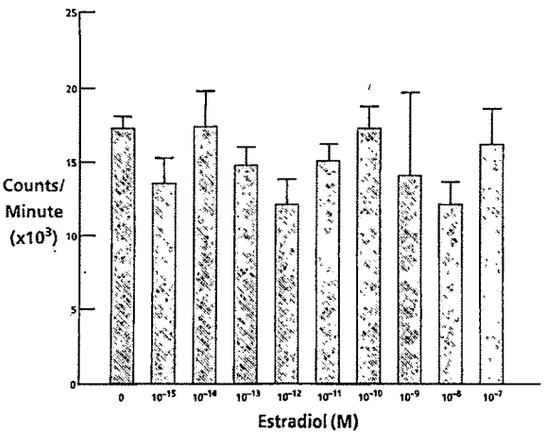


Fig. 7. Effect of addition of estradiol on stromal cell proliferation as measured by [³H]thymidine incorporation (mean ± SEM in counts per minute). Cells were cultured in Nutridoma plus 10% macrophage-conditioned media. Cells were harvested after 48 hours of plating.

study[4] in the rabbit model evaluated the effect of ectopic endometrial implants on peritoneal macrophage number and function. The researchers were unable to demonstrate any effect of the implants on phagocytic cells, suggesting the above theory is in error. Additional studies are currently being conducted in an attempt to ensure that this lack of response is not species specific.

A more intriguing concept is the reverse—that a primary abnormality of peritoneal macrophages results in the promotion of growth of ectopic endometrium.[10] It has been well demonstrated that macrophages secrete growth factors that enhance fibroblast growth and proliferation.[5,6] These factors, cumulatively referred to as macrophage-derived growth factors, have been shown to be distinct from interleukin-1,[13] and may well be composed, at least partially, of platelet-derived growth factor[14] and fibroblast growth factor.[15]

The proliferation process has been studied extensively in the fibroblast. It appears that two types of growth factors are required to create a proliferative response in these cells: a competence factor, which confers on the cell the ability to respond to specific mitogens, and a progression factor, which causes a "competent" cell to progress through the G_1 phase to the S phase of cell growth.[16] Table I provides a partial listing of the substances identified as competence factors or progression factors in the fibroblast. Whereas it is likely that most mesothelium responds in a similar manner, to date this has been inadequately investigated.

Macrophage-derived growth factors function as competence factors in the fibroblast. Evidence presented here indicates a similar role for these growth factors in endometrial stroma. The ability to stimulate stromal growth in the presence of a known progression factor (insulin) in serum-free media suggests that macrophage-derived growth factors can substitute for serum in this competence-producing capacity. Furthermore, the inability of macrophage-conditioned medium to interact with serum in an insulin-free medium to produce cell growth suggests a lack of macrophage progression factor activity. Thus it seems that macrophage-derived growth factor should more appropriately be called macrophage-derived competence factor, a term previously suggested.[17]

Table I. Growth factors in the fibroblast

Competence factor	*Progression factor*
Serum	Endometrial growth factor
Platelet-derived growth factor	Somatomedin C
Fibroblast growth factor	Insulin
Macrophage-derived growth factor	Platelet-poor plasma

The role of estrogen as an endometrial stromal cell mitogen has been well recognized but poorly characterized. Initial studies by Kirk et al.[18] demonstrated an increase in stromal cell growth with 10^{-8} mol/L estradiol in a serum-containing medium. However, Casimiri et al.,[19] using similar conditions, failed to demonstrate any mitogenic effect of estradiol in culture. To further confuse the issue, Fleming and Gurpide[20] showed an inhibition of stromal cell growth with 10^{-8} to 10^{-9} mol/L estradiol in serum- and insulin-containing media. Finally, Irwin et al.[21] recently claimed no mitogenic effect of estrogen in the presence of insulin and serum, yet showed an increase in cell proliferation when estradiol and progesterone were added to the culture simultaneously.

Our studies suggest that estradiol can indeed stimulate endometrial stromal proliferation, but only under specific conditions. Estradiol appears to function as a progression factor in these cells and thus will fail to promote growth in the absence of cell exposure to a competence factor. In addition, estrogen and insulin (and presumably insulin-like growth factors) produce the same progression effect; thus the presence of insulin in culture will eliminate any apparent mitogenic effect of estrogen. Only in otherwise progression factor–free media will growth be apparent after the addition of estrogen.

These facts lead to an intriguing hypothesis for the pathogenesis of endometriosis. Reflux menstruation appears to lead to viable endometrial cells in the peritoneal cavity in the majority of women.[22, 23] However, specific conditions may be required for the tissue to implant and grow. It may well be that the transplanted endometrium fails to develop in the absence of sufficient competence or progression growth factor. However, in women with enhanced macrophage-derived growth factor secretion and relative hyperestrogenism, conditions may be optimal for endometrial implantation and growth. Previous findings of increased macrophage-derived growth factor in the peritoneal fluid of women with endometriosis,[7] as well as the apparent efficacy of hypoestrogenism in combating the disease,[24] make this theory compelling. We believe these findings clearly justify further investigation into these mechanisms as a means of regulating ectopic endometrial cell growth.

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Astrocytes within the hypothalamic arcuate nucleus contain estrogen-sensitive peroxidase, bind fluorescein-conjugated estradiol, and may mediate synaptic plasticity in the rat

David L. Keefe, MD,[a] David S. Michelson,[a] S.H. Lee, MD,[b] and Frederick Naftolin, MD, DPhil[a]

New Haven, Connecticut

Estrogen treatment induces synaptic plasticity accompanied by damaged structures and aggregates of peroxidase in astrocytes in the hypothalamic arcuate nucleus of the rat. Synaptic plasticity also occurs within the arcuate nucleus after physiologic surges of estrogen. Although the function of estrogen-induced peroxidase is unclear at present, in other systems peroxidase can generate free radicals by catalyzing the oxidation of some molecules, including estrogen. Because free radicals underlie remodeling in a number of tissues, estrogen-induced free radicals could mediate synaptic remodeling within the arcuate nucleus. Although they contain estrogen-inducible peroxidase, astrocytes do not contain estrogen receptors as measured by conventional techniques, suggesting that estrogen-inducible peroxidase arises from some novel mechanism. Estrogen could induce peroxidase within receptor-deficient astrocytes by binding to receptors in neurons and stimulating the release of some factor that interacts with astrocytes. Alternatively, estrogen could act directly on astrocytes in the absence of estrogen receptors. Although astrocytes in the hypothalamus of the rat do not contain classical nuclear estrogen receptors, they do bind fluorescein-conjugated estradiol in extranuclear sites. The distribution of fluorescein-conjugated estradiol binding within the hypothalamus overlaps that of peroxidase-rich astrocytes, and double labeling reveals many cells with the stellate morphology of astrocytes, containing both peroxidase and fluorescein-conjugated estradiol binding. However, because peroxidase and fluorescein-conjugated estradiol always occupy different compartments of the cell, the fluorescein-conjugated estradiol is not binding to peroxidase.
(Am J Obstet Gynecol 1991;164:959-66.)

Key words: Astrocytes, estrogen, peroxidase, synaptic plasticity, hypothalamus

Twelve years ago at the One Hundred and First Annual Meeting of the American Gynecological Society meeting Naftolin and Brawer[1] summarized their work on the effects of estrogen in permanently organizing the reproductive brain of the newborn rat during the so called "critical period." They showed that in the male brain androgens are converted to estrogen by aromatase, rendering the male unable to have cyclic gonadotropin release. In the female, the majority of the estrogen made by the ovaries and placenta is bound stoichiometrically to circulating α-fetoprotein and does not enter the brain. In the absence of high levels of estrogen within the central nervous system during the critical period, cyclic gonadotropin release develops in the female as an adult. These studies established the central role of estrogen in organizing the brain of the newborn rat.

The effects of estrogen on the reproductive brain are not restricted to the newborn period. A single large dose of estrogen given to a cycling adult female rat induces persistent vaginal estrus characterized by hypothalamic anovulation with proestrus levels of estrogen and polycystic ovaries.[1-3] Estrogen given continuously at a low level also induces persistent estrus.[4] We now believe that the persistent estrus state that spontaneously arises in middle-aged rats results from the effects of the repeated estrogen surges during the ovarian cycle on the central nervous system.[3] This seems especially likely because estrogen antagonists such as

From the Department of Obstetrics and Gynecology, Yale University School of Medicine,[a] and the Department of Pathology, Hospital of St. Raphael.[b]
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Reprint requests: Frederick Naftolin, MD, DPhil, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT 06510.
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progesterone[5] and pseudopregnancy-pregnancy[5, 6] delay the appearance of senescent constant estrus.

Estrogen induces a tonic pattern of gonadotropin release by disrupting the estrogen-sensitive positive feedback mechanism

The anovulation of naturally or induced persistent estrus rats arises from disruption of the luteinizing hormone–releasing hormone (LH-RH) surge that normally occurs in response to acutely rising levels of estrogen. The same doses of estrogen given acutely to young and aged oophorectomized female rats result in markedly different patterns of LH release[6, 7]: the young, oophorectomized rat responds to estrogen with a robust LH surge. However, with age the LH surge mechanism becomes progressively refractory to triggering by estrogen. Transplantation of ovaries from young into aging female rats does not reverse the anovulatory state, whereas ovaries transplanted from aged into young female rats still can ovulate.[7] These and other related studies suggest that chronic exposure to estrogen alters the sensitivity of the LH-RH surge mechanism to acute administration of estrogen, and that the locus of estrogen's action is in the brain.

The arcuate nucleus of the rat hypothalamus provides an excellent model system for study of estrogen actions on gonadotropin release

Our research focuses on uncovering the cellular and molecular mechanisms involved in estrogen's effects on the hypothalamus. We use the rat model for a number of scientific and logistical reasons. In the rat, areas of the hypothalamus involved in the control of gonadotropin release are separated from each other; LH-RH neurons reside in the preoptic area, whereas many estrogen-sensitive neurons reside in the arcuate nucleus. This enables surgical isolation or dissection of specific nuclei so that they can be studied in great detail. We have focused on the arcuate nucleus of the hypothalamus because: (1) the LH-RH neurons do not bind estrogen, suggesting that estrogen influences LH-RH release indirectly by acting on other neurons,[8] (2) many neurons within the arcuate nucleus contain estrogen receptors[9] and some arcuate nucleus neurons synapse directly on LH-RH neurons[10] in the preoptic area, and (3) studies that use electrolytic lesions, knife cuts, and electrophysiologic stimulation and recording have shown a central role for the arcuate nucleus in mediating estrogen's effects on LH-RH release.[11]

Estrogen influences the morphology of both neurons and neuroglia within the arcuate nucleus

Before discussing further our findings on estrogen actions in the arcuate nucleus, it is helpful to review some fundamental concepts of neuroanatomy. The cellular makeup of the brain consists of two broad classes of cells—neurons and neuroglia.[18] Neurons make synapses with each other where electrical signals in the form of changes in the electrical potential across the neuron's plasma membrane are converted to chemical signals by release of neurotransmitters.[12] These synapses can be identified and counted under the electron microscope. Neuroglial cells have been much less studied than neurons, although they outnumber neurons 10:1 in the brain.[13] Neuroglia formerly were thought of as inactive support cells, but now we know that they can interact with neurons and carry out many important functions on their own. For example, neuroglia can stabilize brain functions by regulating extracellular potassium and hydrogen concentrations,[14, 15] protect brain cells by taking up excess excitatory neurotransmitters such as glutamate,[15] and mold the brain by guiding the migration of neurons during development.[16] In addition, because the foot processes from a single neuroglial cell can envelope and regulate the synaptic environments of widely separated neurons, neuroglial cells may contribute to the synchronization of widely dispersed neurons within the brain.[14, 18] Estrogen affects the morphology of both neurons and neuroglia within the arcuate nucleus.

Estrogen induces synaptic plasticity within the arcuate nucleus

Although estrogen influences many aspects of neuronal morphology, perhaps the most important action of estrogen is on synapses. In adult females estrogen given as an injection or as physiologic surges alters the number of synapses between axons and cell bodies (axosomatic) and between axons and dendrites (axodendritic) within the arcuate nucleus.[17] The number of synapses counted in electron micrographs taken from the arcuate nucleus is decreased by 30% at 8 weeks after a single dose of estrogen. By 32 weeks after estrogen treatment the number of synapses returns to baseline. Careful inspection of electron micrographs of the arcuate nucleus from estrogen-treated rats also shows evidence of synaptic damage, such as degenerating nerve fibers and activated microglia, which appear to be phagocytizing degenerating synaptic boutons.[1-3] Another kind of neuroglia, called astrocytes, also appear to be activated and will be discussed later.

The number of synapses within the arcuate nucleus also varies during physiologic surges of estrogen.[19] The number of synapses counted in the arcuate nucleus of rats killed on estrus is 20% less than that at diestrus, suggesting that rising levels of estrogen on proestrus leads to a reduction in the number of synapses. As estrogen levels decline, the number of synapses returns to baseline. Thus a disconnection and subsequent re-

connection of some neurons occurs within the arcuate nucleus during each ovarian cycle. These findings of synaptic plasticity may contradict our assumptions of the brain as a static tissue (the number of neurons does not change significantly in the adult arcuate nucleus), but recent studies suggest that regulation of synaptic number may occur in other parts of the central nervous system as well.[20]

Estrogen induces peroxidase activity within a class of neuroglial cells called astrocytes

Astrocytes within the arcuate nucleus also change dramatically after estrogen treatment—they develop large, electron-dense inclusions.[1-3] Originally called "gliosomes" they bear a striking morphologic resemblance to organelles called peroxisomes.[21] Peroxisomes use molecular oxygen and hydrogen peroxide to carry out oxidative reactions.[21] Like peroxisomes, gliosomes contain peroxidase activity.[22, 23] To determine whether gliosomes are peroxisomes, we are studying whether gliosomes contain other oxidative enzymes found in peroxisomes.

Peroxidase activity within the arcuate nucleus is sensitive to estrogen because estrogen treatment increases,[22, 23] and oophorectomy decreases[23] peroxidase within the arcuate nucleus. Arcuate nucleus neuroglial peroxidase behaves like peroxidases located in other estrogen-responsive tissues such as the breast, uterus, cervix, and vagina.[24, 25] The induction of arcuate nucleus peroxidase by estrogen is so robust and consistent that we[23] and others[24, 25] have used it as a marker of a tissue's estrogen responsiveness.

Recently we found that estrogen-sensitive peroxidase is not confined to the arcuate nucleus. When we surveyed serial sections through the entire brains of two rats for the presence of peroxidase activity with the 3,3′-diaminobenzidine reaction, we found that after the arcuate nucleus, the highest concentration of peroxidase activity resided in the periventricular nucleus. A number of other nuclei involved in the regulation of gonadotropin release such as the suprachiasmatic nucleus, medial preoptic area, and median eminence had minimal peroxidase activity.[23] When we compared peroxidase activity within the periventricular nucleus and arcuate nucleus of estrogen-treated, cycling, and oophorectomized rats, we found peroxidase was estrogen-sensitive in both of these nuclei. On the other hand, peroxidase activity within the posterior cingulate cortex, an area lacking estrogen receptors, was not estrogen-sensitive. It is of interest that the distribution of estrogen-inducible neuroglial peroxidase in the arcuate nucleus and periventricular nucleus closely overlaps that of estrogen receptor-positive catecholaminergic neurons that have been implicated in neuroendocrine regulation,[26] raising the possibility of some interaction between these peroxidase-rich neuroglia and estrogen-receptor positive catecholaminergic neurons. Furthermore, because estrogen induces peroxidase activity in the periventricular nucleus, it would be of interest to determine whether it induces synaptic remodeling in the periventricular nucleus as well.

Whereas peroxidases may protect cells from the harmful effects of free radicals by breaking down hydrogen peroxide, they can also contribute to oxidative stress by catalyzing the oxidation of some molecules including estrogen and catecholamines

We do not completely understand the function of estrogen-inducible neuroglial peroxidase and its relationship to estrogen-induced synaptic plasticity, but we can make some generalizations on the basis of studies carried out in other systems. Peroxidases are present to varying degrees in every cell, where they protect the cells from the harmful effects of hydrogen peroxide and associated free radicals produced during oxidative reactions.[21] In addition, peroxidase can generate free radical intermediates by catalyzing the oxidative metabolism of some molecules including estrogen[27, 28] and catecholamines.[29] Thus under different conditions peroxidase can inhibit or promote the formation of free radicals.

A free radical is a molecule with an unpaired electron in its outer orbit, making it highly unstable and quite reactive.[30] Free radicals form from oxygen during normal aerobic cellular metabolism, as well as during the oxidative metabolism of a number of cellular constituents such as estrogen and catecholamines.

Although free radicals generated during oxidative reactions threaten to damage cellular constituents, cells use a number of defensive strategies to contain the toxic effects of free radicals. First, a variety of enzymes including various peroxidases and superoxide dismutase catalyze the breakdown of free radicals and hydrogen peroxide.[30] In some species intracellular oxidative stress itself induces the synthesis of peroxidases and antioxidant enzymes. This effect is mediated by a protein whose oxidized but not reduced form activates transcription of peroxidase and antioxidant genes.[38] Second, peroxidases,[39] superoxide dismutase,[40] and many oxidizing enzymes[39, 40] have signal sequences that target them to peroxisomes or mitochondria. Within these specialized compartments, enzymes that generate peroxide and free radicals remain in close proximity to enzymes that break down these toxic species. Finally, a number of antioxidants including vitamins E and C and carotenes scavenge oxygen free radicals in the cell's hydrophobic and hydrophilic domains.[31]

Whereas most cells contain the toxic effects of free radicals with these defensive strategies, other cells har-

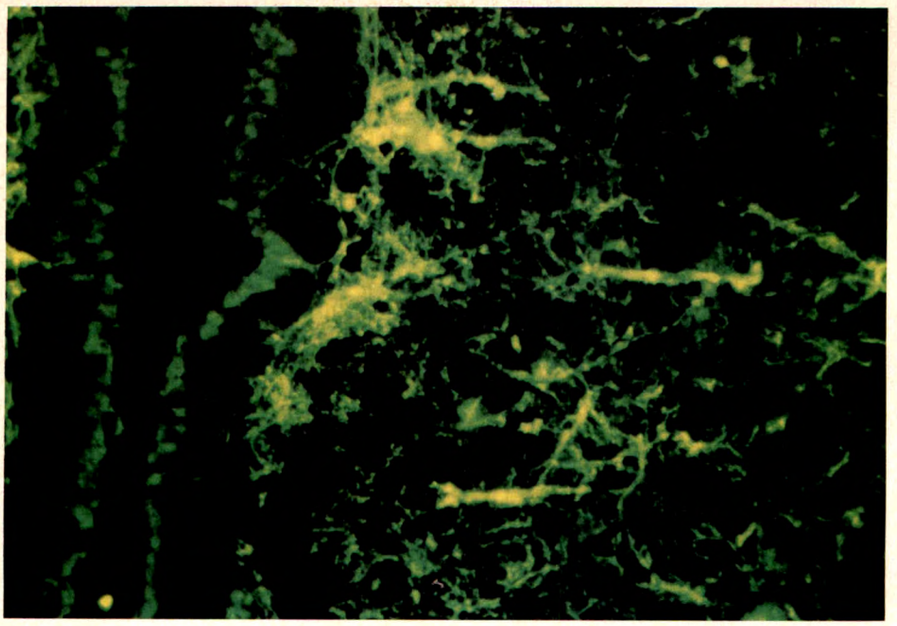


Fig. 1. This epifluorescence micrograph shows binding of fluorescein-conjugated estradiol to stellate cells within the arcuate nucleus of the rat hypothalamus. Cells stained with the fluorescein-conjugated estradiol appear green-yellow in these 10 μm-thick cryostat sections. The third ventricle can be seen on the left-hand side of the photomicrograph. The distribution of fluorescein-conjugated estradiol binding overlaps that of astrocytes that contain estrogen-inducible peroxidase.

ness these toxic molecules to serve specialized functions (e.g., leukocytes and macrophages use free radicals during the immune response,[41] and the tissue remodeling in the postfertilization envelope in the oocyte of some species[42] and the regressing corpus luteum[32, 33] involve free radicals). Free radicals also may bring about pathophysiologic damage to the central nervous system such as in ischemic reperfusion injury[42] and Parkinson's disease.[43]

In addition to breaking down hydrogen peroxide, peroxidases can catalyze a number of reactions that produce free radicals as intermediates. These include the metabolism of estrogen through the catechol estrogen pathways, which produce reactive quinones and semiquinones,[36, 37] and the oxidation of catecholamines by monoamine oxidase.[44]

Estrogen-induced peroxidase and free radical production provide a hypothetical mechanism to explain the molecular basis of estrogen-induced synaptic plasticity

We do not understand how estrogen, peroxidase, and free radicals relate to synaptic remodeling within the arcuate nucleus. However, the information from the rapidly growing field or free radical biology summarized in this article allows us to construct hypotheses that can be tested experimentally. One possible hypothesis is that estrogen induces synaptic remodeling independent of any effects on peroxidase or free radicals, and that the neuroglial activation observed within the arcuate nucleus represents only a reaction to tissue damage. Alternatively, estrogen could directly induce synaptic damage in the arcuate nucleus through its reactive oxidative metabolites. In this hypothesis, oxidative metabolism of estrogen catalyzed by peroxidase initiates a vicious cycle in which free radical intermediates that arise during the oxidative metabolism of estrogen induce further synthesis of peroxidase, resulting in further production of free radical intermediates. In this way estrogen provided by physiologic surges or pharmacologic administration could produce free radicals, induce peroxidase, and lead to synaptic damage.

Although estrogen induces peroxidase in neuroglia, conventional assays have never demonstrated estrogen receptors in these cells

Although estrogen induces peroxidase activity in neuroglial cells within the arcuate nucleus and periventricular nucleus, neuroglial cells themselves do not contain estrogen receptors as determined by autoradiography[45] or in situ hybridization with an oligonucleotide probe for the estrogen receptor.[46] A number of mechanisms could account for estrogen inducibility of peroxidase in the absence of estrogen receptor. First, even in the absence of estrogen receptors, oxidative

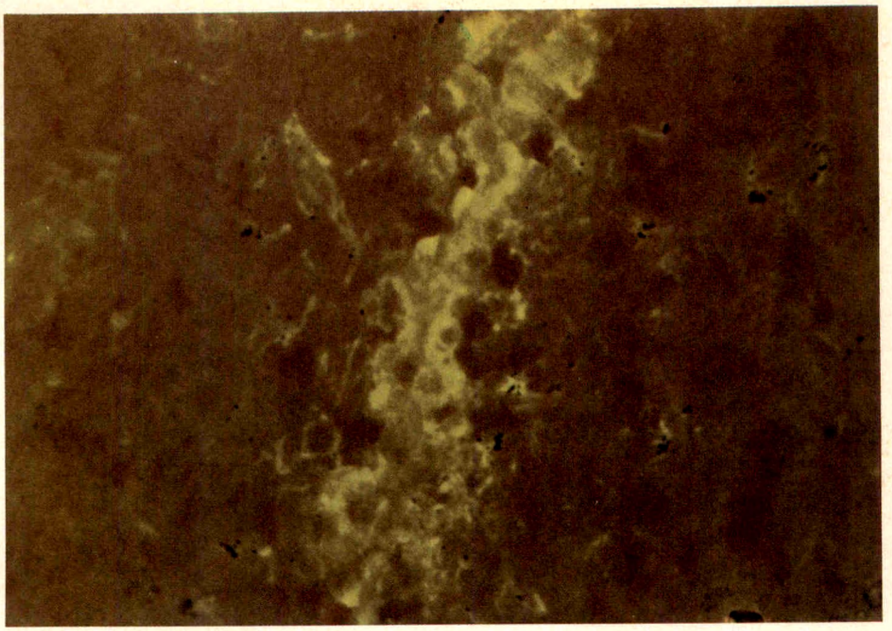


Fig. 2. This epifluorescence micrograph shows that many cells within the arcuate nucleus both contain peroxidase as measured by 3,3′-diaminobenzidine *(black granules)* and bind fluorescein-conjugated estradiol *(green-yellow)*.

metabolism of estrogen might directly induce peroxidase activity in neuroglial cells by increasing intracellular oxidative stress.[38] Alternatively, estrogen could bind to estrogen receptors in neurons and induce the secretion of some factor that induces peroxidase in surrounding astrocytes by paracrine action. One candidate for mediating such a neuron-neuroglial interaction is nerve growth factor, which induces peroxidase activity in PC-12 cells in culture.[47] A third possibility is that estrogen might act directly on neuroglial cells in some novel estrogen receptor–independent manner. Tsibris et al.[48] recently described a family of proteins with structures that share homology with the estrogen-binding domain of the estrogen receptor. Furthermore, he showed that estrogen can influence the enzymatic activity of at least one of these proteins in vitro.[48] This and other data[49] suggest that estrogen does not always require the presence of its classical receptor in a cell to influence that cell's function.

Peroxidase-rich neuroglial cells in the arcuate nucleus and periventricular nucleus of the hypothalamus bind fluorescein-conjugated 17 β-estradiol

Although neuroglial cells do not themselves contain nuclear estrogen receptors, they do bind estrogen as measured by an in vitro fluorescein-conjugated 17 β-estradiol (estradiol-bovine serum albumin-fluorescein isothiocyanate) histochemical technique,[50] which depends on permeabilitization of cells with freezing or detergents. This material reliably identifies breast cancer cells that are also receptor-positive, as determined by immunohistochemistry with a monoclonal antibody for the estrogen-receptor, and it predicts the efficacy of sex steroid manipulation therapy on breast cancer.[51] However, because it identifies nonnuclear binding,[50, 51] its relationship to the estrogen receptor is unclear.

Because a preliminary communication had suggested binding of this reagent to astrocytes,[52] and because we had found estrogen-inducible peroxidase activity in neuroglial cells that lacked classical estrogen receptors as measured by conventional methods, we used the in vitro fluorescein-conjugated estradiol histochemical assay to look for estrogen binding in cryostat sections of the female rat hypothalamus. We found almost all the fluorescein-conjugated estradiol confined to cells with the morphology of astrocytes (Fig. 1). Staining was especially prominent within the arcuate nucleus and periventricular nucleus, but was clearly different from the peroxidase (3,3′-diaminobenzidine reaction), which was also present. As reported previously in breast and uterus,[50, 51, 53] the probe did not label nuclei, but instead was confined to extranuclear sites in a distribution consistent with either cell cytoplasm or membrane.

Because labeled cells had the same morphology as astrocytes and appeared most numerous within the arcuate nucleus and periventricular nucleus in the same distribution as peroxidase-rich astrocytes, we looked for the presence of peroxidase activity and fluorescein-conjugated estradiol in the same cells. First we cut 10

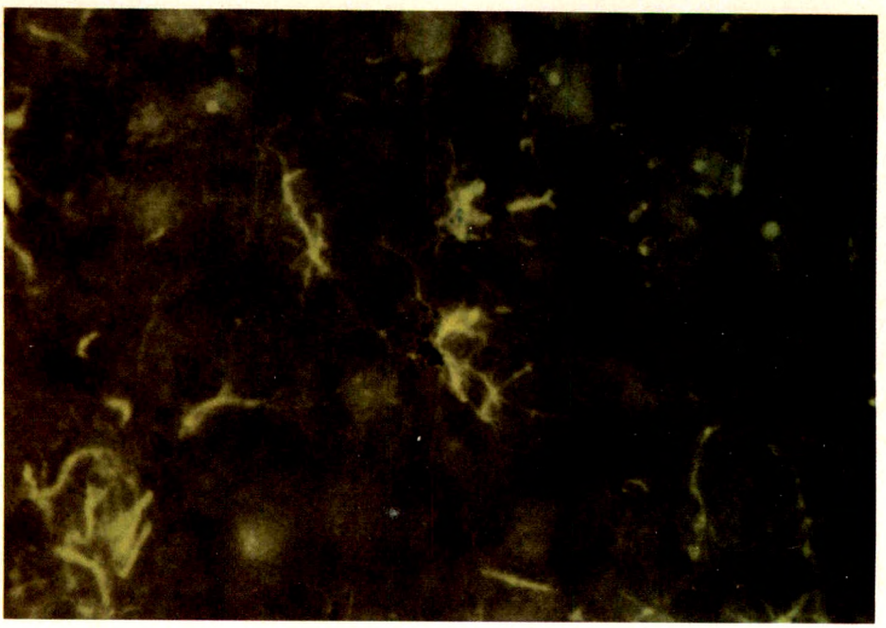


Fig. 3. Although many stellate-shaped cells in the arcuate nucleus both contain peroxidase and bind fluorescein-conjugated estradiol, the two labels appear in different compartments of the cell. Peroxidase, as indicated by the presence of 3,3′-diaminobenzidine granules, appears as distinct, dark granules, whereas fluorescein-conjugated estradiol binds widely throughout the cell. Thus fluorescein-conjugated estradiol appears to bind to neither nuclear receptors nor to peroxidase, but rather to some constituent of either cytoplasm or membrane.

μm-thick cryostat sections from the hypothalamus of estrogen-treated female rats. Then we stained these sections with the fluorescein-conjugated estradiol reagent. After fixing the sections in paraformaldehyde, we stained the sections for peroxidase activity using the 3,3′-diaminobenzidine reaction. We found many cells with the morphology of astrocytes that contained both 3,3′-diaminobenzidine granules and fluorescein-conjugated estradiol, and the distribution of these cells again overlapped that of peroxidase-rich neuroglia (Fig. 2). Although some cells appeared to contain either peroxidase activity or fluorescein-conjugated estradiol alone, inspection of the remainder of these cells in other sections frequently revealed the presence of both labels.

Although peroxidase and fluorescein-conjugated estradiol frequently appeared in the same cells, they always occupied different compartments within the cell (Fig. 3). 3,3′-Diaminobenzidine appeared as small, discrete cytoplasmic granules, whereas the fluorescein-conjugated estradiol labeling appeared homogeneous, suggesting that either it binds throughout the entire cytoplasm or that it labels some membrane component. Given the separate intracellular locations of peroxidase and fluorescein-conjugated estradiol, the fluorescein-conjugated estradiol clearly must not bind to peroxidase itself. Further studies will need to characterize and identify the fluorescein-conjugated estradiol-binding substance(s). In particular, it will be interesting to learn whether the estrogen-binding substance shares homology with the nuclear estrogen receptor or to estramustine binding protein,[54] another substance that binds a congener of estradiol and predicts response of some tumor cells to hormonal therapy. In the meantime, the reagent provides a fluorescent probe that should prove useful to identify these astrocytes for future morphologic studies.

Comment

In summary, estrogen induces synaptic plasticity and neuroglial peroxidase activity within the arcuate nucleus and periventricular nucleus of the rat hypothalamus. The finding of estrogen-induced peroxidase activity outside of the arcuate nucleus suggests the need for further studies of estrogen-induced synaptic plasticity in other nuclei. Estrogen-induced peroxidase activity could cause or result from synaptic plasticity, although recent studies that show that peroxidase activity and associated free radicals cause tissue damage and remodeling in a number of physiologic and pathologic situations support the former hypothesis. Furthermore, although estrogen induces peroxidase activity in neuroglial cells, neuroglia have never been found to bind estrogen by autoradiography or to contain the message for estrogen receptor by in situ hybridization.

We have identified binding of fluorescein-conjugated estradiol within these peroxidase-rich, but estrogen receptor–deficient neuroglial cells. Because the molecular basis of this binding remains controversial, future studies will need to further characterize and identify the substance that binds fluorescein-conjugated estradiol in neuroglia. These results raise questions about the role of peroxidase-rich neuroglia in the metabolism of estrogen and provide a fluorescent probe to identify them for future morphologic studies.

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The role of the placenta in nutrition of the human fetus

Henning Schneider, MD
Berne, Switzerland

Bidirectional transport across the placenta provides substrates required for fetal nutrition and prevents accumulation of metabolites in the fetal compartment. Transmission of compounds across the placental barrier occurs by diffusion and carrier-mediated transport. The technique of dual in vitro perfusion of an isolated cotyledon has provided important data for the human placenta. The high degree of permeability for hydrophilic molecules was recently confirmed by in vivo measurements that allow estimates of flux across the human placenta for a number of compounds. The bidirectional nature in transplacental exchange requires asymmetry in flux from the maternal to the fetal side or vice versa, which can be assured by a concentration gradient or an active transport system. There is increasing evidence that placental metabolism with active modification of the composition of nutrients released to the fetus in addition to transport plays an important role. (AM J OBSTET GYNECOL 1991;164:967-73.)

Key words: Human placenta, bidirectional transport, in vitro perfusion, permeability

The human fetus together with the placenta, its supply organ, for a long time have been quite inaccessible to the investigator. Concerns for both fetal and maternal safety and ethical considerations placed severe restrictions on invasive research in human pregnancy. The bulk of information on fetal and placental physiology was obtained in animal experiments.

New technologies such as real-time ultrasonography together with Doppler ultrasonographic techniques have given us a direct view into the inside of the fetal world and provided us with new information on fetal and placental growth and blood flow in the major vessels. Blood sampling in the human fetus can be performed under almost undisturbed steady-state conditions at various stages of gestation, which seemed impossible only a few years ago. These new tools of research provide additional information, rapidly expanding the knowledge in particular on the human fetus and the human placenta.

It is obvious that the placenta plays a central role in fetal nutrition, and there is no survival of the fetus in utero without support by the placenta. Whereas the association of placental transport function and fetal nutrition is self-evident, other functional aspects, such as placental hormone synthesis and various metabolic activities, are also closely linked to the supply of nutrients to the fetus. Placental proteohormones secreted into the maternal bloodstream act as signals sent from the conceptus to the mother to induce important changes in the maternal cardiovascular system and in basic metabolism, which are needed to guarantee substrate flow through the placenta. Energy metabolism is not only needed to support the synthetic process, but also to maintain active transport across the barrier that separates maternal and fetal blood. Furthermore, there is increasing evidence that the placenta does not restrict itself to the simple transmission of the mixture of substrates that is presented by the maternal bloodstream, but there is continuous modification of the composition of nutrients through the metabolic activity within the placenta. This seems especially relevant to the supply of amino acids because essential amino acids of the branched chain structure, such as leucine, are to some extent converted and released into the fetal bloodstream as nonessential neutral straight chain amino acids such as alanine and glycine.[1,2]

Because placental transport function is affected by several factors, it should be discussed as an integral part of the maternal and fetal organism (Table I). Because of its easy accessibility, isolated human placental tissue was widely used to overcome some of the restrictions placed on research in human pregnancy. Any type of experiment performed with isolated placental tissue creates an artificial situation and can only provide part of the answer. Several techniques have been described, ranging from membrane vesicles with the use of only fractions of cellular material, villous preparations and slices to dual perfusion of either the whole organ or a single cotyledon. Important contributions were made by these techniques. Perfusion, which as an integrated system retains a good deal of the complexity of the intact organ, seemed particularly promising. Among other attractions it is the simplicity of the system, al-

From the Department of Obstetrics and Gynecology, University of Berne.
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Reprint requests: Henning Schneider, MD, Universitäts-Frauenklinik, Schanzeneckstrasse 1, Berne, Switzerland CH-3012.
6/6/27588

Table I. Factors that affect placental transport function

Uterine-umbilical blood flow
Permeability of the barrier
Carrier systems, asymmetry of transport
Placental metabolism, energy supply
Maternal metabolism/fetal metabolism, transplacental gradient

lowing control of experimental variables, that leads to answers that are difficult to obtain in the complex in vivo set up. In this article, after a brief description of the perfusion technique, the validity of the approach will be discussed and some examples of the type of information on human placental transport derived from in vitro perfusion experiments will be presented. Some of these data are more relevant since new information on human placental transport obtained in vivo is available for direct comparison.

In vitro perfusion of human placental tissue

Technique. The technique for dual-perfusion of an isolated cotyledon was developed over 25 years ago and was repeatedly described in detail.[3, 4] Placentas are obtained immediately post partum. After selection of a suitable cotyledon, the supplying vessels are identified on the chorionic plate and cannulated for perfusion of the fetal vascular system of that particular cotyledon. The intervillous space is separately perfused via blunt cannula penetrating the decidual plate and the two perfusion circuits are maintained, one for the maternal compartment corresponding to the intervillous space and one for the fetal vessels inside the villi. The preparation is fixed in a temperature-controlled perfusion chamber and various parameters such as pressure, flow rates, temperature, pH, and gas partial pressures are monitored throughout the experiment.

Validity of in vitro perfusion. There was a great deal of questioning as to the validity of the data when they were first presented. Apart from unphysiologic conditions of any in vitro set up, the significant time lag between interruption of blood flow and initiation of in vitro perfusion was of serious concern. Over the years several investigators accumulated substantial evidence on the morphologic and functional integrity of the perfused tissue as shown by the continuation of hormone synthesis, active carrier-mediated transport, and the maintenance of the ultrastructure.[5-10]

The placenta, similar to early embryonic and other poorly differentiated tissues, has a higher degree of resistance to ischemia than mature tissues of similar metabolic activity as shown by the preservation of tissue adenosinetriphosphate after ischemia.[11] The significance of this general concept for in vitro placental perfusion has been shown by an on-line assessment of synthesis of energy-rich phosphates under conditions of various degrees of hypoxia including ischemia with magnetic resonance spectroscopy. After the start of in vitro perfusion, an increase in tissue adenosinetriphosphate with a simultaneous fall in inorganic phosphate could be shown and adenosinetriphosphate levels were maintained for up to 10 hours of perfusion. After total interruption of both perfusion circuits for 2 to 4 hours a complete recovery of adenosinetriphosphate generation could be shown with resumption of perfusion.[12]

Permeability to hydrophilic molecules in vitro

Passive diffusion across the placenta is an important mechanism for substrate exchange. Diffusional flux in the direction from the mother to the fetus contributes to fetal nutrient supply, whereas flux in the reverse direction allows for clearance of the fetal compartment from metabolites and other compounds. Limitation to diffusion is given by the resistance within the tissue layers interposed between fetal and maternal blood, which is significant for hydrophilic molecules. This is in contrast to lipid-soluble substances that show a much higher degree of permeability, with uterine and umbilical blood flows being the limiting factors for transplacental flux rates.[13] In the human placenta there are two layers that contribute to diffusional resistance, the trophoblast that covers the villi and the endothelium that lines the fetal capillaries inside the villi. Hydrophilic molecules have to find their way through water-filled channels that extend through the syncytiotrophoblast and communicate with intercellular channels in the endothelial layer.[14]

Large differences among species have been shown for placental permeability to hydrophilic molecules that must be explained by differences in the size of the water-filled channels. In the sheep the barrier is particularly tight with no diffusional flux for hydrophilic substances with a molecular weight > 400 daltons.[15] In contrast, the guinea pig placenta is much more permeable, allowing diffusion even for inulin with a molecular weight of >5000 daltons.[16] For smaller molecules such as mannitol the permeability of the guinea pig placenta is even 30 times higher. Whereas similarity in structure would suggest that the human placenta has a similar degree of permeability for hydrophilic molecules, some controversy remains.[17]

Evidence of the permeability of the human placenta comes from in vitro perfusion experiments and lately also from in vivo determinations performed during cesarean section. With the in vitro approach a wide range of different molecules that varied in size between 60 and 5000 daltons were studied. Labeled test compounds were added to the perfusion fluid circulating through the maternal compartment. By measuring the

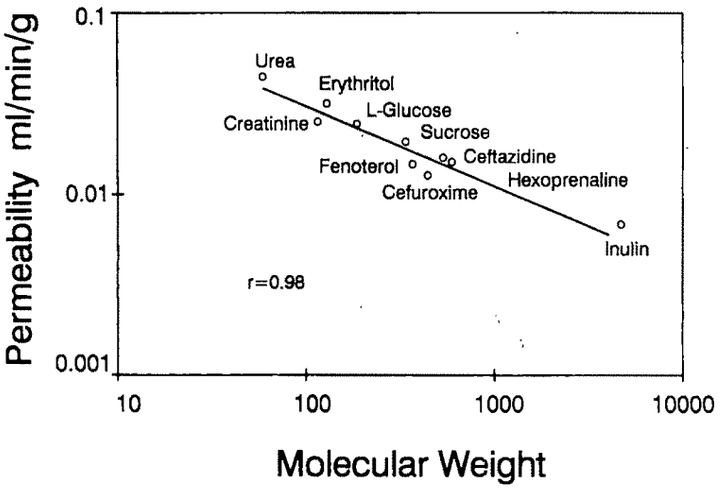


Fig. 1. Correlation between placental permeability and molecular weight for several hydrophilic compounds as determined by in vitro perfusion of an isolated human placental cotyledon. Data points for some compounds were taken from Schneider H, Sodha RJ, Prögler M, Young MPA. Permeability of the human placenta for hydrophilic substances studied in the isolated dually in vitro perfused lobe. Contrib Gynecol Obstet 1985;13:98-103.

flow rate in the fetal compartment together with the concentration of the test compound in fetal venous samples, the flux per minute and gram placental tissue can be calculated, from which the permeability is derived as the ratio of the flux over the gradient. Details of the experimental design have been described.[18] There is a close correlation between permeability and molecular weight over the studied range, indicating that free diffusion in water is the determining factor and that the water-filled channels are wide enough to allow unlimited passage for all hydrophilic molecules up to 5000 daltons (Fig. 1).

These data on the permeability of the human placenta for lipid insoluble solutes could be of great interest. Most electrolytes, urea, many drugs and peptides belong in this group. Over a wide range of different molecular sizes there is no steric hindrance within the human placental barrier, and diffusion is inversely proportional to molecular size. A good part of endogenous and exogenous hydrophilic compounds, which are of general interest in human pregnancy, fit in this range, and from the plot shown in Fig. 1 a permeability value can be taken for any substance. For clinical purposes and discussions in physiology, the flux or the amount of substance getting across the fetus is of more relevance than the permeability and this can be derived from the permeability, provided the transplacental gradient is known. With more and more information on fetal blood levels coming from samples obtained by cordocentesis, estimates on transplacental gradients can be performed and flux can be calculated as a product of permeability times transplacental gradient. A direct comparison of the flux values with figures on fetal requirements or fetal production will be of interest in particular for endogenous compounds and will indicate whether diffusional transport is adequate for supply or clearance or whether active transport or placental metabolism are involved as additional mechanisms.

Permeability to hydrophilic molecules in vivo

A principal question is to what extent does the experimental situation of in vitro experiments change the permeability characteristics of the barrier? Experiments in the guinea pig have shown that in situ perfusion yields higher figures on permeability than those in the intact animal and the discrepancy seems to be larger with increase in molecular size.[19] Recent data on human placental permeability determined at the time of cesarean section allow comparison between in vitro and in vivo results.[20-22] The two groups performed permeability studies of the human placenta in vivo using a similar approach that consisted of infusion of test molecules into women scheduled for elective cesarean section starting about 1 hour before surgery. Samples were taken from the anticubicle vein at the opposite arm at 20-minute intervals, whereas cord blood and amniotic fluid were obtained at the moment of delivery. The amount transferred to the fetus was determined either by collection of all the urine excreted by the newborn up to 50 to 60 hours after delivery or by use of the concentration in cord blood and a factor for the volume of distribution in fetal tissues. The mean transplacental gradient that is also needed to calculate per-

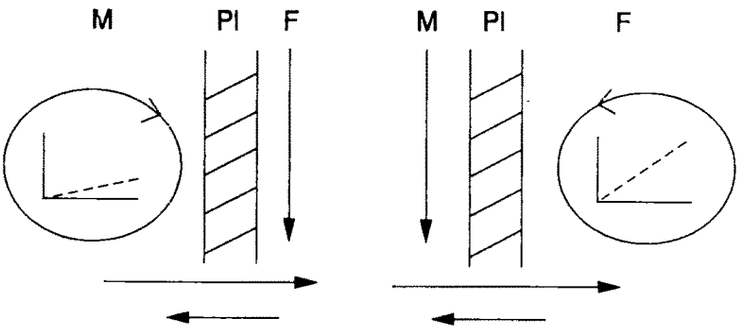


Fig. 2. Experimental design for study of asymmetry of flux across the in vitro perfused human placenta. *M, F,* maternal, fetal perfusion circuit; *Pl,* placental membrane.

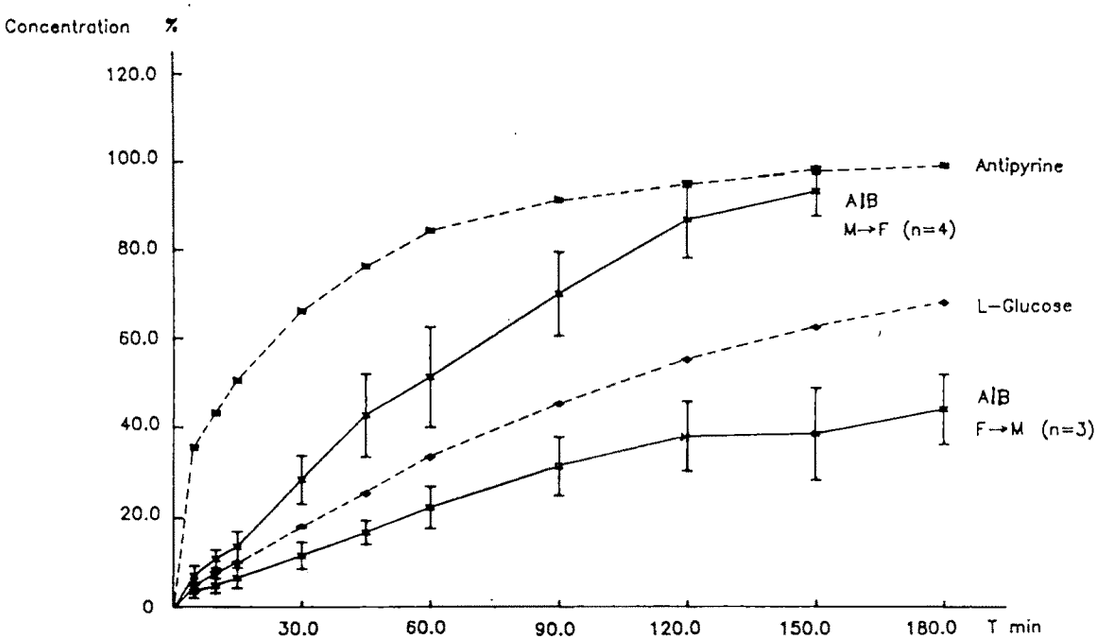


Fig. 3. Asymmetry in transplacental flux of α-aminoisobutyric acid from maternal to fetal *(M → F)* and fetal to maternal *(F → M)* direction. The flux of the reference compound antipyrine and L-glucose showed no difference in the two directions. From Schneider H, Proegler M, Sodha R, Dancis J. Asymmetrical transfer of alpha-aminoisobutyric acid (AIB), leucine and lysine across the in vitro perfused human placenta. Placenta 1987;8:141-51.

Table II. Human placental permeability to hydrophilic molecules

	Molecular weight	*In vivo*	*In vitro*	*Ratio (in vitro : in vivo)*
Mannitol/L-glucose (ml/min/gm)	182/180	0.012* ± 0.004	0.023 ± 0.005	2.1
Inulin (ml/min/gm)	5200	0.0014* ± 0.0002	0.006 ± 0.002	4.6
		0.009†		0.7

*Data from Bain MD, Copas DK, Landon MJ, Stacey TE. In vivo permeability of the human placenta to inulin and mannitol. J Physiol (Lond) 1988;399:313-9.

†Data from Thornburg KL, Burry KJ, Adams AK, Kirk EP, Faber JJ. Permeability of placenta to inulin. Am J Obstet Gynecol. 1988;158:1165-9.

meability was taken from the area under the concentration curve derived from maternal samples and the cord blood sample. Because of the limited number of data points in particular for the fetal concentration curve, this can only lead to an estimate of the transplacental gradient.

Permeability values obtained by this direct in vivo measurement for mannitol and inulin together with the values obtained in vitro are shown in Table II. Whereas the in vivo values found for mannitol and inulin permeability by a group from London are smaller than the in vitro results, there is a fairly good agreement between in vitro and in vivo data as reported by a group from Portland.[21, 22] Possible explanations for the difference in results in the two in vivo studies have been discussed.[22] In view of the great individual variations shown in both in vivo studies, the data on permeability obtained by in vitro perfusion and in vivo measurements are not too different and can be used to give estimates of human placental permeability of any hydrophilic molecule within the described range. Both approaches—in vivo and in vitro—show a much higher permeability than what has been described for the four-layered sheep placenta. The in vivo permeability for mannitol, a molecule of the same size as glucose, is close to 60 times higher in the human placenta than in the sheep.[15]

It should be stressed at this point that the discussion of passive diffusion has been restricted to hydrophilic molecules and other factors such as partial lipid solubility, protein binding or carrier-mediated transport will lead to completely different rates of flux.

Asymmetry of flux

With consideration of the rather high degree of leakiness of the hemochorial placenta even for hydrophilic molecules, there is more or less free exchange of many compounds between maternal and fetal blood. Net flux of nutrients, electrolytes, vitamins, and other compounds from the mother to the fetus is needed to secure a constant supply for fetal energy metabolism as well as tissue growth and differentiation. Clearance of metabolites and end products, on the other hand, require net movement from the fetus to the mother. Directional flux with asymmetry in flux rates is a basic principal of transplacental substrate exchange. This asymmetry may be a result of a downhill gradient with net movement from the side of higher to lower concentration, or preferential transport may be provided by an active transport system independent of differences in concentration. The asymmetry of flux by carrier-mediated transport has also been studied in the in vitro perfused placenta.[8, 23]

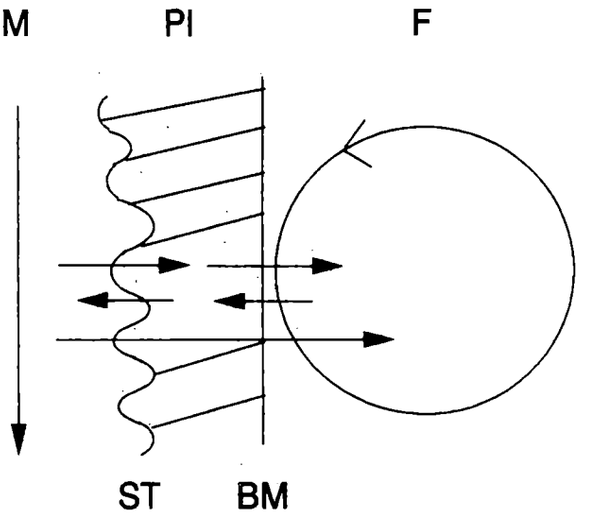


Fig. 4. Asymmetry in uptake and efflux at the maternal and fetal surface leading to asymmetry in transplacental flux. *M, F,* maternal, fetal perfusion circuit; *PI,* placental barrier; *ST,* syncytiotrophoblast; *BM,* basal membrane.

For this type of study a special experimental design with one perfusion circuit run as an open system and the other side recirculated has been applied (Fig. 2). The test compound under investigation is added to the perfusate on the open side, providing a constant level with no test substance present in the recirculating compartment. The change in concentration as a result of the appearance of test compound in the recirculating compartment allows calculation of the rate of flux. With the possibility of changing open and closed sides in the same or different experiments, the net flux in the two directions may be compared. As an example, the transfer rates of radioactively labeled α-aminoisobutyric acid is shown in Fig. 3. It is compared with two passively diffusing reference compounds: antipyrine, a small liquid-soluble molecule with rapid diffusion, and L-glucose. Both antipyrin and L-glucose show identical transfer when measured in the two directions, therefore only one concentration curve in the recirculating compartment is shown. The difference in transfer rate between antipyrine and L-glucose is a result of the difference in solubility, with antipyrine being highly lipid soluble whereas L-glucose is a purely hydrophilic molecule. α-Aminoisobutyric acid shows a clear asymmetry with a higher flux from the mother to the fetus. It is interesting that the back flux from the fetal to the maternal side is even lower than that of L-glucose, although α-aminoisobutyric acid is a smaller molecule, suggesting that there is less back flux for the amino acid as would be expected by passive diffusion. Ex-

traction of perfused tissue at the end of the experiment showed accumulation of the amino acid in the tissue with no significant difference in the two types of experiments. Similar differences in flux could be shown for representatives of other amino acid transport systems such as leucine or lysine.[8]

Comparison among three different amino acids showed a significantly higher transfer rate for leucine than for α-aminosobutyric acid or lysine, confirming results from animal experiments that showed that transfer of neutral branched chain amino acids is faster than that of neutral straight chains such as alanine or glycine as well as basics such as lysine.[24, 25]

Whereas the asymmetry in flux across the placenta seems to be an important mechanism in transport and has been shown for water-soluble vitamins such as riboflavin,[23] the mechanism that explains the difference in flux in the two directions is not completely understood. The kinetic equilibria at the maternal and fetal surface of the placenta are of a complex nature and are involved not only in the development of gradients between the bloodstream and the tissue but also in the transplacental gradient expressed as the difference between maternal and fetal concentration (Fig. 4). A rapid uptake of the compound at the maternal surface with the microvillous covering of the syncytiotrophoblast seems to be of primary importance. At this interface uptake greatly exceeds substrate release and a cotransport of amino acids with sodium dependent on sodium/potassium–adenosinetriphosphatase activity has been shown for human placental membrane vesicles prepared from the microvillous surface.[26, 27] There is some evidence for uptake of amino acids from the fetal side as well as could be shown in the isolated guinea pig placenta.[28] The asymmetry of uptake and efflux at both interfaces between maternal blood and trophoblast and fetal blood and capillary endothelium are contributing to transplacental transport. Whereas uptake and efflux on both sides are involved in the transplacental flux of neutral amino acids, the uptake of the acidic amino acid glutamic acid, which was shown from maternal and fetal blood, predominantly serves placental metabolism because there is no net flux across the placenta in either direction.[29, 30]

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Pulmonary injury associated with antepartum pyelonephritis: Can patients at risk be identified?

Craig V. Towers, MD, Carol M. Kaminskas, MD, Thomas J. Garite, MD, Michael P. Nageotte, MD, and Wendy Dorchester, MPH
Orange and Long Beach, California

The development of pulmonary injury in cases of antepartum pyelonephritis is rare but serious. To date, factors that might identify patients at risk have not been determined. We compared 11 patients with pyelonephritis and pulmonary injury with 119 patients with pyelonephritis only. Pulmonary injury was more likely to occur in the more severe cases; however, the presence of a maternal heart rate >110 beats/min and a fever to 103° F 12 to 24 hours before the occurrence of respiratory symptoms in a gestation >20 weeks was highly predictive of pulmonary injury. The most significant predictive factors associated with pulmonary injury were elements of treatment such as fluid overload, use of tocolytic agents, and, to a lesser extent, choice of antibiotic. Therefore, if tocolytic agents are considered at all in the management of acute pyelonephritis in pregnancy, they should be used only in patients with documented cervical change. In addition, urinary output should be monitored very closely. These data also may suggest a cause of the pulmonary edema that is occasionally seen in the management of premature labor with the use of tocolytic agents and fluids in the presence of a possible occult infection. (Am J Obstet Gynecol 1991;164:974-80.)

Key words: Pyelonephritis in pregnancy, pulmonary insufficiency in pregnancy, tocolytic complications

Adult respiratory distress syndrome is a well-known risk that is associated with sepsis[1] but occurs relatively rarely during pregnancy.

Pyelonephritis complicated by pulmonary injury was first reported in four patients in 1984 by Cunningham et al.[2] They postulated that the injury was endotoxin induced, which caused alveolar-capillary injury leading to respiratory injury. Two other individual cases were reported[3, 4] in patients with similar histories.

In 1987 Cunningham et al[5] reported on 15 antepartum patients, identified during a 7-year period, who had respiratory insufficiency with pyelonephritis. These patients were compared with 30 matched control subjects. A higher incidence of multisystem disorders, such as tachycardia, hemolysis, etc., was found in those patients in whom pulmonary injury ultimately developed. However, no clinical risk factors that could be used on admission to predict pulmonary injury were identified.

The purpose of this study was to review our experience with pyelonephritis to determine if any risk factors could be isolated to identify patients at risk for pulmonary injury.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of California at Irvine, and the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Long Beach Memorial Womens Hospital.
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Reprint requests: Craig V. Towers, MD, 2801 Atlantic Ave., P.O. Box 1428, Long Beach, CA 90801-1428.
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Material and methods

All cases of antepartum pyelonephritis treated at the University of California Irvine Medical Center and Long Beach Memorial Womens Hospital from Jan. 1, 1985, to Dec. 31, 1988, were identified by medical record computer files. Pyelonephritis was defined as a positive urine culture with ≥100,000 cfu/ml of a single species of bacteria,[6] maternal fever ≥38° C (100.4° F), and flank pain with tenderness on examination. All three findings, in addition to a viable pregnancy, were necessary for inclusion of patients in the data analysis.

These cases were reviewed for historic, clinical, and treatment factors. Historic factors included gravidity, parity, gestational age, prenatal care, and a history of pyelonephritis. When clinical factors are evaluated, mean values are not very helpful in the identification of predictive parameters. Therefore a modified step analysis was used on each variable to determine cutoff points. Clinical factors included maximum maternal heart rate, maximum maternal respiratory rate, maximum temperature, days febrile (≥38° C) after admission, blood transfusion, presence of renal or ureteral stone, microorganisms cultured, and elevated serum creatinine level. Treatment factors included use of tocolytics, fluid management, and choice of antibiotic.

Pulmonary injury was characterized by dyspnea, tachypnea, documented hypoxemia with a need for

Table I. Risk of pulmonary injury with antepartum pyelonephritis with respect to historic factors

Factor	*Pyelonephritis without pulmonary injury*	*Pyelonephritis with pulmonary injury*	*p Value*
Mean gravidity	2.46 ± 1.63	1.73 ± 0.94	0.41
Mean parity	0.94 ± 1.19	0.55 ± 0.97	0.71
Prenatal care	31/119	4/11	0.59
Gestational age >20 wk	84/119	11/11	0.036
History of pyelonephritis	6/119	1/11	0.35

Table II. Clinical factors in determining the risk of pulmonary injury with antepartum pyelonephritis

Factor	*Pyelonephritis without pulmonary injury*	*Pyelonephritis with pulmonary injury*	*p Value*
Maternal heart rate* ≥ 110 beats/min	13/119	11/11	<0.00001
Respiratory rate >28 breaths/min	4/119	11/11	<0.00001
Fever ≥103° F (first 24 hr)	13/119	9/11	<0.00001
≥3 Febrile days in hospital	16/119	10/11	<0.00001
Transfusion needed	0/119	4/11	<0.00001

*Heart rate 12 to 24 hours before symptoms of respiratory insufficiency occurred.

supplemental oxygen, and an abnormal chest radiograph consistent with pulmonary edema or adult respiratory distress syndrome.

All collected information was placed in a computer data file. The χ^2 test with Yates' correction and Fisher's exact test were used in statistical analysis. A p value of ≤ 0.05 was considered significant. All tests were considered against a two-sided alternative hypothesis. Factors that were potentially predictive of patients who were destined to have pulmonary injury were further subjected to multivariate analysis.

Results

A total of 214 cases of pyelonephritis were identified. Eighty-four were excluded because the patients did not meet all four criteria for having antepartum pyelonephritis. No case of pulmonary injury was seen in these 84 excluded cases. Eleven cases of pulmonary injury (8.5%) that met all four criteria occurred in the remaining 130. This allowed for comparison of 11 cases of pyelonephritis and pulmonary injury with 119 cases of pyelonephritis without pulmonary injury. Pulmonary injury occurred within 36 to 72 hours of admission in all 11 cases.

Gravidity, parity, prenatal care before admission, and a history of pyelonephritis were not significantly correlated with pulmonary injury (Table I). However, pyelonephritis with pulmonary injury occurred significantly later in gestation with all cases occurring after 20 weeks' gestation.

Clinical factors were highly significant in correlating pyelonephritis with pulmonary injury (Table II). A maternal heart rate of ≥110 beats/min and a maximum temperature of ≥103° F (39.4° C) that occurred 12 to 24 hours before the onset of respiratory symptoms were very important. Maximum respiratory rate was evaluated at several intervals after admission and before pulmonary injury occurred. A respiratory rate of ≥28 beats/min, which was significant when compared with the maximum respiratory rate of the control group, occurred only when pulmonary injury was already apparent. No difference in the maximum respiratory rates of the two groups was found in the 12- to 24-hour period preceding the onset of pulmonary symptoms. Therefore the respiratory rate was not predictive of pulmonary injury.

The need for transfusion and the number of febrile days in the hospital also were significantly correlated with pulmonary injury. However, because all cases of pulmonary injury were apparent within 3 days of admission, the duration of fever also was not predictive. In addition, the four patients in whom transfusion was required received the units of blood after the diagnosis of pulmonary injury was made.

A serum creatinine level was obtained within 12 hours of admission in 89 of the patients. Only eight patients had a value of ≥1.0 mg/dl. In three of these patients pulmonary injury developed. The difference in the incidence of an elevated creatinine level was not significant ($p = 0.10$) between the two groups. *Esche-*

Table III. Antibiotics used as first line of treatment for first 48 hours after admission

Antibiotic used	*Cases with pulmonary injury*	*Cases without pulmonary injury*	*Total*
Ampicillin only	6	31	37
Cephalosporin only	5	72	77
Gentamicin only (or with ampicillin or cephalosporin)	0	14	14
Tobramycin	0	1	1
Trimethoprim-sulfamethoxazole	0	1	1
TOTAL	11	119	130

Table IV. Treatment factors and risk of pulmonary injury with antepartum pyelonephritis

Factor	*Pyelonephritis without pulmonary injury*	*Pyelonephritis with pulmonary injury*	*p Value*
Tocolytic use	10/119	7/11	<0.00001
Fluid balance*			
>2 L	32/119	7/11	0.017
>3 L	11/119	5/11	0.004
Ampicillin only as initial treatment	31/119	6/11	0.075

*Fluid balance calculated over the first 48 hours in cases without pulmonary injury and calculated until the occurrence of pulmonary injury in 11 affected cases.

richia coli was the microorganism cultured in 106 cases, 7 of which incurred pulmonary injury. Three of 14 cases in which *Klebsiella* was the infecting organism had pulmonary injury, and *Enterobacter* was identified in the final case of pulmonary injury. The microorganism identified was not significant ($p = 0.19$).

Table III shows the antibiotics used as the first line of treatment for 24 to 48 hours after admission. The choice of antibiotic was not significant, although there was a trend toward a higher risk of pulmonary injury with ampicillin only. Culture sensitivities indicated that only two of the 11 cases with pulmonary injury were resistant to the first-line antibiotic. Seventeen cases were culture resistant to the first-line antibiotic in the 119 cases without pulmonary injury; this finding was not significant ($p = 0.5$). Unfortunately, blood cultures were not routinely obtained, especially in the cases in which pulmonary injury did not develop. However, only one of the 11 cases with pulmonary injury had a positive blood culture; in the other 10 cases blood cultures were negative.

Table IV, which evaluates the treatment factors, reveals that fluid overload and the use of tocolytics were the most highly significant factors in the development of pulmonary injury. The fluid intake rates were similar in both groups; however, urinary output was less in the cases that ultimately developed pulmonary injury (Table V). Because pulmonary injury occurred 36 to 72 hours after admission, the mean fluid intake (via intravenous infusion, medications, and oral routes) was calculated on an hourly basis until pulmonary injury was detected. The mean hourly urinary output in these 11 cases was calculated in the same manner. In the 119 cases in which pulmonary injury did not develop, the mean hourly fluid intake and urinary output were calculated for the first 48 hours after admission. In addition, the difference in fluid intake and urinary output also was significant between the two groups.

In nine of the 17 cases in which tocolytic agents were used, magnesium sulfate was given (pulmonary injury occurred in four of these cases), and eight patients received terbutaline (three of these eight experienced pulmonary injury); this also was not significant ($p = 0.58$).

A multivariate analysis with the use of logistic regression was then performed on the data. Because the purpose of this article was to identify risk factors that predicted respiratory insufficiency, a model using only factors that were present before the pulmonary injury was created. This model included gravidity, parity, gestational age, prenatal care, a history of pyelonephritis, microorganisms cultured, maternal heart rate of ≥110 beats/min, temperature ≥103° F in the first 24 hours after admission, use of a tocolytic agent, choice of antibiotic, fluid overload >2 L, and fluid overload >3 L. The final model resulted in five significant factors with a multiple R^2 of 0.59. These factors, in order of significance, were a maternal heart rate of ≥110 beats/min that was present 12 to 24 hours before respiratory symptoms occurred, the use of a tocolytic agent, the use of ampicillin only as the first line of treatment, a temperature ≥103° F in the first 24 hours, and fluid overload >3 L.

In three patients with pulmonary injury placement of a Swan-Ganz catheter was necessary. All values were consistent with adult respiratory distress syndrome with normal to elevated cardiac output (8.0, 8.1, and 10.9 L/min) and normal to slightly elevated pulmonary capillary wedge pressures (11, 14, and 18 mm Hg). All

Table V. Comparison of mean fluid intake and urinary output between the two groups

*Fluid status**	*Pyelonephritis without pulmonary injury (N = 119)*	*Pyelonephritis with pulmonary injury (N = 11)*	*p Value*
Mean fluid intake (ml/hr)	173.2 ± 26.8	166.3 ± 34.3	0.44
Mean urinary output (ml/hr)	112.7 ± 24.4	82.5 ± 21.8	0.002
Difference in intake and output (ml/hr)	60.4 ± 20.4	83.8 ± 30.0	0.001

*Fluid status calculated during the first 48 hours in the cases without pulmonary injury, and fluid status calculated up to the occurrence of pulmonary injury in 11 affected cases.

patients with pulmonary injury had arterial blood gas analysis with P_{O_2} values of ≤65 mm Hg.

In this study there were 127 patients, three of whom had had pyelonephritis twice; this resulted in a total of 130 cases. Twenty-eight patients were lost to follow-up, and one patient underwent termination of her pregnancy. Six (6.1%) of the remaining 98 patients were delivered before 36 weeks of gestation; the remaining patients were delivered at term. Two of the preterm deliveries were in the pulmonary injury group; both had premature rupture of membranes. The risk of preterm delivery was not significant when compared between the two groups ($p = 0.13$). Finally, only two patients had renal stones, and one of these patients was in the pulmonary injury group.

Comment

The incidence of pulmonary injury in our patient population was unexpectedly high (8.5%) when compared with the data of Cunningham et al.[5] Several factors might explain this variation. The study by Cunningham et al. reported on 15 cases found during a 7-year period during which 750 cases of pyelonephritis occurred. The incidence of pulmonary injury was therefore 1 in 50 cases of antepartum pyelonephritis (2%). However, not all 750 cases were reviewed to ensure adherence to the strict criteria required for a diagnosis of pyelonephritis. Therefore some cases might have been excluded (as they were in our evaluation), which would result in a smaller denominator and an overall higher incidence of pulmonary injury. Indeed, the cases excluded from our study were those of less severe illness. In addition, Cunningham et al. compared the 15 cases with 30 matched controls and did not find fluid management to be a factor. Also, tocolytic agents were not used. In our study two cases of pulmonary injury did occur when tocolytics were not used and when fluid overload was not present. This would suggest a baseline risk of 1 in 65 cases (1.5%), which is consistent with the data from the study by Cunningham et al. However, if fluid management is not closely monitored and if tocolytics are used in such patients, the incidence of pulmonary injury can be markedly increased, as this study indicates. Therefore it is important for clinicians to realize that a relatively benign disorder can be transformed into a very severe illness from iatrogenic causes if close monitoring is not observed.

It was somewhat amazing to see the high fluid intake measured on an hourly rate (Table V) in the management of these cases. This high fluid intake was probably instituted on the premise that part of the treatment regimen for pyelonephritis is to "flush" the kidney. For the most part, this management was tolerated by the patients in this study. However, if urinary output does not stay in line with the intake rate, then the patient is at high risk for incurring pulmonary injury. Thus prudent management of fluid intake and output is essential.

All 17 previously published cases,[3-5] as well as the 11 cases in this study, were >20 weeks of gestation, except for one case, which was at 19 weeks. This suggests that the occurrence of pulmonary injury as a result of pyelonephritis is dependent on gestational age.

Our study does reveal that pulmonary injury is more likely to occur in the more severe cases of antepartum pyelonephritis as indicated by highly significant clinical factors. The study by Cunningham et al.[5] confirms this with respect to tachycardia, tachypnea, febrile state, hemolysis, etc. However, the presence of maternal renal insufficiency that was significant in Cunningham's article was not significant in this study when it was evaluated in the first 24 hours of admission as a potential predictor of pulmonary injury. The development of pulmonary injury with *E. coli* pyelonephritis was 6.6% (7/106) versus 21.4% (3/14) with *Klebsiella* pyelonephritis; this was not significant. However, this may not have reached statistical significance as the result of a β-error in our study population.

The use of ampicillin only as initial treatment was highly implicated as a risk factor in the development of pulmonary injury, although results of the study were not conclusive. Therefore an aminoglycoside might be added to the initial treatment plan, especially in the treatment of the more severe cases of antepartum pyelonephritis.

The pathophysiologic parameters of pulmonary injury in the majority of our cases were probably multifactorial. Sepsis can cause alveolar capillary injury, but

the degree of capillary injury may vary from mild to severe. The addition of excess fluid can result in increased pulmonary capillary wedge pressure and in decreased oncotic pressure, and the addition of tocolytics raises cardiac rate, which increases left atrial pressure, as well as pulmonary venous pressure. Thus, in cases of milder degrees of alveolar capillary injury that would not ordinarily result in pulmonary edema, the addition of β-sympathomimetics or magnesium sulfate and excess fluid could cause frank pulmonary edema. The cardiac output and pulmonary capillary wedge pressure in each of our three patients who underwent invasive hemodynamic monitoring support this suggested mechanism.

The clinical implications of the information presented in this study are relatively straightforward for patients with pyelonephritis, but the data also may increase our understanding of the mechanism for at least some cases of pulmonary edema that are associated with tocolytic therapy for premature labor. The majority of reports describing pulmonary edema in patients with premature labor involve the use of β-sympathomimetic agents; however, pulmonary edema has been reported with the use of magnesium sulfate as well. Most patients in whom pulmonary edema develops do not have underlying cardiac disease and, while they often are given excess fluids, fluid overload alone generally does not explain the severity of the response. Reports of these cases have not suggested a cardiogenic cause of the pulmonary edema.[6-8] Furthermore, experimental trials in animals have failed to reproduce pulmonary edema with fluid overload and the use of β-sympathomimetic agents without also inducing a capillary permeability defect.[9, 10] After having reviewed all the reported cases of pulmonary edema associated with premature labor and tocolytic therapy, Benedetti[11] suggested that the majority of the patients in whom this complication developed might also have an unrecognized septicemia in association with an occult infection, such as occult chorioamnionitis. Therefore he contended that the apparent pulmonary edema in those cases was secondary to pulmonary injury caused by undiagnosed sepsis. The information obtained from the patients in this study supports Benedetti's argument, because the risk of pulmonary injury was markedly increased if tocolytics were used and if the patients had fluid overload.

In summary, tocolytic agents should be used only to treat the contractions of those pregnant patients with pyelonephritis who have documented and significant cervical change. The perceived benefit of tocolytic therapy must be weighed against the significant risk of pulmonary injury. Futhermore, strict management of fluids should occur so that patients do not have fluid overload. The warning signs of high fever (≥103° F) and a maternal tachycardia (≥110 beats/min) during the first 24 hours of admission are highly significant risk factors and should be closely monitored, especially if the patient is >20 weeks of gestation.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. STANLEY A. GALL, Louisville, Kentucky. The authors have reviewed the clinical course of patients who have been diagnosed with acute pyelonephritis without pulmonary injury and have compared them with a smaller group of patients with acute pyelonephritis pulmonary injury as manifested by adult respiratory distress syndrome. The main question asked of the study is whether patients at risk for adult respiratory distress syndrome can be identified by clinical factors.

The authors were careful to select patients that constituted a homogeneous group by requiring four findings to be present. These included a positive urine culture of a single species of bacteria, maternal fever of ≥38° C, and flank pain with tenderness on examination. Eighty-four of 214 patients (39%) with a diagnosis of acute pyelonephritis were excluded because they did not meet all four criteria. These were appropriate exclusions because no cases of adult respiratory distress syndrome occurred in the excluded group and the excluded group had milder disease. Thus 119 of 130 patients (91.5%) with acute pyelonephritis and without adult respiratory distress syndrome were compared

with 11 of 130 patients (8.5%) with acute pyelonephritis and in whom adult respiratory distress syndrome occurred. It is important to note that the definition of pulmonary injury required the presence of dyspnea and tachypnea, the need for supplemental oxygen, the presence of hypoxemia, and an abnormal chest radiograph consistent with adult respiratory distress syndrome or pulmonary edema.

The authors report that gravidity, parity, the presence of prenatal care before admission, and a history of pyelonephritis were not significantly related to adult respiratory distress syndrome; however, all patients with adult respiratory distress syndrome were >20 weeks of gestational age. The clinical factors found to be highly significant were a maternal heart rate ≥110 beats/min and a maximum temperature of ≥39.4° C that occurred 12 to 24 hours before the onset of respiratory symptoms. The iatrogenic factors that were highly significant for adult respiratory distress syndrome included tocolytic use and excess fluid balance of >2 and >3 L. There was a trend toward significance with ampicillin as the only therapy.

The use of tocolytic agents in 17 of 130 patients (13%) with acute pyelonephritis resulted in the antibiotic treatment of 7 of 11 patients (64%) with adult respiratory distress syndrome; 4 of 9 (44%) received magnesium sulfate and 3 of 8 (37.5%) received terbutaline. The authors state that intravenous infusion rates were similar in groups with and without adult respiratory distress syndrome but that urinary output was less in the group with adult respiratory distress syndrome.

It seems to follow that the patients in whom acute pyelonephritis developed were those with a maternal heart rate of ≥110 beats/min 12 to 24 hours before the respiratory symptoms and who were treated with a tocolytic agent, received ampicillin as first-line chemotherapeutic agent, had a temperature of ≥39.4° C in the first 24 hours after admission, and had fluid overload >3 L. Study data indicated that adult respiratory distress syndrome with acute pyelonephritis was identified in 7 of 130 patients (5.3%) when tocolytics were used and in 2 of 130 patients (3%) when tocolytics were not used. It is unclear what happened to the remaining 2 of 11 patients (18%) with adult respiratory distress syndrome, because four received magnesium sulfate, three received terbutaline, and two did not received a tocolytic agent. Therefore my first question concerns what the other two patients received.

My additional question addresses the use of criteria of a pulse of 110 beats/min and a temperature of 39.4° C. Were the total intravenous infusions on a daily basis different among the seven patients who received tocolytic agents and in whom adult respiratory distress syndrome developed as compared with those who did not receive tocolytic agents and in whom the syndrome did not develop?

It would be of additional value to us if the fluid balance studies were explained more completely. The authors report that the total intravenous fluid infusions were similar in the two groups of patients but that the urinary output was lower in the group with adult respiratory distress syndrome, even though cardiac output was increased and serum creatinine level was normal. Is there an explanation for this unusual observation?

The authors are to be commended for using strict clinical criteria and for carefully analyzing this important disease entity. Their conclusions that clinical and iatrogenic factors can predict patients who are at high risk for development of adult respiratory distress syndrome are justified. I feel that this is a significant advance in our understanding of the disease entity of adult respiratory distress syndrome as a complication of acute pyelonephritis of pregnancy.

Dr. Ronald S. Gibbs, Denver, Colorado. I have three questions. The first involves the 84 exclusions that represent approximately 39% of the patients. First of all, how many patients, if any, were excluded because of colony counts <100,000 CFU/ml (a usual standard) but who may have had colony counts >100 CFU/ml?

Stamm reported that 100 CFU/ml was actually a more sensitive measure than 100,000 CFU/ml in symptomatic individuals.

Second, are the risk factors still the same if all 84 exclusions are added back in?

Third, was the identification of particular organisms related to pulmonary injury? I think that the Dallas group had reported *Klebsiella* as an associated organism. It was not found in this study, but the numbers seem to indicate that >20% of the *Klebsiella* isolates were associated with pulmonary injury as compared with an association of only about 5% of the *E. coli* isolates.

Dr. Thomas H. Kirschbaum, Los Angeles, California. I would like to ask the authors to comment on the certainty with which they can make this diagnosis without measuring the pulmonary capillary wedge pressure. We find that pulmonary capillary wedge pressure is commonly elevated in patients in whom we suspect the diagnosis. These patients do get better as a result of diuresis, and they may have additional endothelial injury but we can't be certain.

Dr. David A. Eschenbach, Seattle, Washington. It's fairly apparent that, with respect to pathophysiologic characteristics, either lipopolysaccharide or other material from the organism is causing some effect on the lung. A couple of iatrogenic problems such as tocolytics and fluid overload, have been identified. Could ampicillin therapy be another iatrogenic problem, in the sense that about 25% or more of urinary isolates are somewhat resistant to ampicillin?

Is it possible to determine whether the patients in whom pulmonary injury developed were those who had isolates that were resistant to ampicillin, which suggests that an inability to effectively treat the infection was an additional factor?

Dr. Robert K. Creasy, Houston, Texas. There seemed to be some question about exactly which injury the patients really experienced. There are some similarities that can be drawn with pulmonary edema treated with tocolytics alone. For example, it's relatively rare to see a patient experience pulmonary edema within the first 24 hours of treatment with a tocolytic

agent. The patients in this study were, I believe, experiencing pulmonary edema within 36 to 72 hours after tocolytic treatment. This is the time span within which most cases of pulmonary edema associated with tocolytics occur.

I think it's also relatively well established now that fluid overload predisposes patients receiving tocolytic therapy to pulmonary edema, and indeed a number of the patients in this study had significant overload.

The next question is whether pulmonary edema leads to adult respiratory distress syndrome, which has been shown to occur with tocolytic treatment alone, or does the patient really just have adult respiratory distress syndrome?

Dr. Garite (Closing). I appreciate the comments from Dr. Gall and the discussion. In response to Dr. Gall's questions, I apologize that the information was somewhat unclear. Seven of the 11 patients in whom pulmonary injury developed had had tocolytic treatment with or without excess fluid. Two had excess fluid only, and two had neither risk factor. I meant to imply that two of the 11 patients had neither risk factor when I made that statement.

There are two answers to the questions about the necessity of cutoff points and the reasons that we selected cutoff points with clinical factors that are predictive of pulmonary injury, such as tachycardia or fever. First, we felt that the purpose of this study was to determine whether there were clinically predictive risk factors. Therefore choosing a cutoff seemed more appropriate than choosing averages that a clinician could use to ascertain a need for concern about the patient's condition. We did a form of stepwise analysis that examined cutoff points found only infrequently. If you review the table that examines clinical factors, you will note that all cutoff points chosen (for example, high fever or tachycardia) occurred in $<10\%$ of the total population.

Total intravenous fluids were indeed similar in patients who had pulmonary injury and in those who did not have pulmonary injury, at least up until the mean of 48 hours.

The observation about oliguria is very cogent, especially as it pertains to patients with normal or elevated cardiac output with accompanying normal creatinine levels. Let me clarify that point. We examined creatinine on admission for two reasons. First, we were looking at the predictive value of a clinical entity. Second, in $>95\%$ of all patients included in the study, a creatinine level was determined, so this was a useful parameter. However, only patients with pulmonary injury or another complication had a repeat creatinine measurement, so it wasn't fair to compare the subsequent test result.

Half of the patients with pulmonary injury had an elevation in creatinine level >1.0 after pulmonary injury. So, as Cunningham and others have mentioned, patients often have multisystem disease; possibly endotoxin damages the endothelium within the glomerulus, which results in oliguria.

I would also like to respond to Dr. Gibbs' question about the 84 patient exclusions. Approximately half of these exclusions were as a result of our having isolated multiple organisms in the urine or as a result of colony counts $\leq 10^5$ Cfu/ml. Many patients were excluded because of lost urine cultures that occurred predominantly at the public institution, and very few patients were excluded because of absence of other clinical factors.

The risk factors were not analyzed, so I cannot answer that question.

The interesting observation in Cunningham's study that concerned *Klebsiella* as a more predominant cause of pulmonary injury than *E. coli* was not found in our study. In Fisher's exact test or a χ^2 test analysis, I can't remember the numbers specifically but the p value demonstrated a slight but not significant trend. If there is a trend, it certainly would require larger numbers; I can't comment on that further.

Dr. Kirschbaum questioned the certainty of the diagnosis without pulmonary monitoring. Of course, this is a very important point. First, cardiac disease must be ruled out, and the level of fluid overload must be assessed. Fluid overload can be determined in part by clinical response, because patients with adult respiratory distress syndrome tend to have clearance at a slower rate than do patients with simple fluid overload. Fluid overload also can be determined in part by a chest radiograph, but there is certainly a debate over this.

Beyond that, I think it just makes clinical sense that pulmonary edema is unlikely to occur from minor degrees of fluid overload in the presence or absence of cardiac disease.

Dr. Eschenbach commented on the sensitivity of antibiotics in the treatment of pulmonary injury. He questioned whether the organisms created the pulmonary injury and, if so, if these organisms were insensitive to ampicillin. Some were. I don't have the exact numbers, but somewhere between a third and a half were insensitive. That's about what we would expect with the sensitivity of coliforms to ampicillin in our hospital. With that small number, it's hard to speculate more than we already have, but we did consider this, although we did not include the data in our manuscript.

Finally, Dr. Creasy commented on the similarities of treatment with tocolytics in patients with preterm labor who did not have pyelonephritis. Those similarities were extremely striking to us as well. The similarities in timing of the onset of the disease and the similarities in the pulmonary and cardiac monitoring data in patients who have had invasive monitoring are quite tremendous. I think that most patients require some additional insult beyond mild pulmonary capillary injury to cause them to have frank pulmonary edema.

In a review of seminars in pulmonary medicine in 1986, it was commented that probably 80% to 90% of cases of pulmonary injury or adult respiratory distress syndrome are not clinically apparent but are transient. Unless patients have some other injury, such as overwhelming sepsis, shock, or trauma, they don't manifest pulmonary injury, and this may indeed be the case with tocolytic treatment and fluid overload.

Randomized comparative trial of indomethacin and ritodrine for the long-term treatment of preterm labor

Richard E. Besinger, MD, Jennifer R. Niebyl, MD, William G. Keyes, MD, PhD, and Timothy R.B. Johnson, MD

Baltimore, Maryland

A randomized prospective trial was performed to compare the efficacy and safety of ritodrine and indomethacin in the long-term treatment of preterm labor. Forty patients with intact membranes in preterm labor at 23 to 34 weeks' gestation were randomized to receive either intravenous ritodrine or oral indomethacin as the first-line tocolytic agent. Successful intravenous ritodrine therapy was followed by oral terbutaline therapy, and indomethacin-treated patients continued to receive oral indomethacin. Treatment failures were defined as progressive preterm labor or patient intolerance, and these patients were treated with intravenous magnesium sulfate. Ritodrine and indomethacin were equally successful in delaying preterm birth as defined by interval to delivery, gestational age at delivery, delivery delayed >7 days, attainment of 35 weeks of gestation, percentage of patients who required magnesium sulfate therapy, percentage of patients who were readmitted with premature rupture of membranes, absence of recurrent preterm labor, and infant birth weight. More than 80% of mothers who received ritodrine voiced complaints of β-sympathomimetic side effects, and one patient discontinued treatment as the result of intolerance. There were minimal patient complaints with indomethacin use. No statistically significant differences were noted in neonatal outcome as defined by Apgar scores, umbilical cord pH, intensive care days, ventilator days, or neonatal deaths. However, three cases of primary pulmonary hypertension were observed in the indomethacin group. We had not previously observed this problem with short-term (24 to 48 hours) indomethacin therapy. (Am J Obstet Gynecol 1991;164:981-8.)

Key words: Indomethacin, ritodrine, tocolysis

Inhibition of preterm labor remains a major therapeutic dilemma in modern-day obstetrics. Ritodrine hydrochloride is the only tocolytic agent that is currently approved by the Food and Drug Administration for this use. However, the clinical use of intravenous β-sympathomimetic therapy is somewhat limited by maternal side effects, development of tolerance, or lack of efficacy. Magnesium sulfate also has been widely used as a tocolytic agent. Prostaglandin synthetase inhibitors have been proposed as an effective alternative and often are used in cases in which either ritodrine or magnesium sulfate has failed.

The placebo-controlled studies with indomethacin have demonstrated an inhibitory effect on preterm labor,[1, 2] and one comparative trial of short-term therapy has shown efficacy equivalent to that of ritodrine.[3] In two additional randomized trials, the therapeutic effect of indomethacin was evaluated in combination with concurrent ritodrine therapy.[4, 5] In both studies the combined ritodrine-indomethacin treatment group had a significant prolongation of pregnancy when compared with that of the group treated with ritodrine plus placebo or with the group treated with ritodrine alone.

However, adverse effects in the neonate that result from the use of indomethacin have been of concern. Human and animal studies have shown transient ductal constriction,[6] although no long-term adverse effects on infants have been noted. Case reports have suggested an association between indomethacin treatment and primary pulmonary hypertension in the neonate,[7] but confounding factors such as sepsis or perinatal hypoxia may have contributed to the difficult neonatal adaptation in these cases. Primary pulmonary hypertension did not occur in the series reported with <48 hours of indomethacin treatment.[1, 3, 8-10]

The purpose of this randomized trial was to evaluate the efficacy and side effects of indomethacin compared with ritodrine for initial tocolysis and long-term treatment of preterm labor.

Material and methods

Forty-three patients who were between 23 and 34 weeks of gestation and who were treated at The Johns Hopkins Hospital or the Francis Scott Key Medical Cen-

From the Divisions of Maternal-Fetal Medicine and Neonatology, The Johns Hopkins Hospital and the Francis Scott Key Medical Center.

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Reprint requests: Richard E. Besinger, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153.

6/6/27367

ter for an initial episode of preterm labor with intact membranes were recruited between March 1987 and September 1988 for this study. This protocol was approved by the Joint Committee on Clinical Investigation at The Johns Hopkins University School of Medicine. All participating patients provided informed consent after receiving both written and oral presentations of the risks and benefits of each tocolytic agent to be used. Three randomized patients (7%) were excluded from this analysis. Two patients randomized to ritodrine therapy were eliminated before delivery as a result of protocol errors. One patient assigned to indomethacin therapy was eliminated soon after enrollment because of clinical, laboratory, and ultrasonographic evidence of a concealed abruptio placentae. The data for the remaining 40 patients were analyzed.

Patients qualified for the protocol if they were <34 weeks of gestation and had preterm contractions at a rate of at least 4 in 20 minutes or 8 in 60 minutes during the initial observation period. In addition, documented cervical change by a single examiner, initial cervical dilatation >2 cm, or effacement >75% was required for inclusion in the study. Subjects were excluded from the study if they presented with cervical dilatation >4 cm, or with rupture of membranes or if they had a cervical cerclage in place.

All participating subjects had cervical cultures taken for gonorrhea, group B streptococcus, chlamydia, and mycoplasma. Each subject underwent an ultrasonographic examination that included an amniotic fluid index.

Each patient was randomly assigned by a table of random numbers via a sealed envelope to either ritodrine or indomethacin treatment. Patients in the ritodrine group received the drug intravenously at a rate of 100 to 350 μg/min until contractions ceased. Maintenance intravenous fluids were administered at a rate of 125 ml/hr. Ritodrine was continued for 8 to 12 hours in the delivery suite after cessation of contractions before oral β-sympathomimetic therapy was started. Oral terbutaline was given in doses necessary to prevent recurrent uterine activity, titrated to a maternal heart rate of 80 to 100 beats/min, ranging from 2.5 to 5.0 mg every 4 to 6 hours. The choice of oral terbutaline as a maintenance tocolytic agent was made solely as a result of cost considerations.

Patients assigned to the indomethacin group received 125 ml/hr of maintenance fluid and were given an initial oral loading dose of 50 mg of indomethacin. Then indomethacin 25 to 50 mg was given orally at 4-hour intervals until contractions ceased; this protocol was continued for maintenance therapy at 25 mg orally every 4 to 6 hours. The initial treatment time spent by the indomethacin group in the labor and delivery suite was kept similar to that of the ritodrine group, in spite of different routes of drug administration.

In cases of successful initial tocolysis, study subjects were subsequently transferred to the antepartum ward and remained hospitalized on modified bed rest for an average of 2 to 3 days. A repeat four-quadrant amniotic fluid index was obtained before discharge in subjects receiving indomethacin; this fluid index was obtained weekly after discharge. All tocolytic agents were discontinued at 35 weeks of gestation.

Initial treatment failure was defined as progressive preterm labor that occurred in spite of maximum drug dosage or as patient intolerance that necessitated discontinuation of the original tocolytic agent. These patients then received intravenous magnesium sulfate with an initial bolus of 4 gm, which was followed by a continuous infusion at a rate of 2 to 4 gm/hr, and the initial tocolytic was stopped. Magnesium sulfate infusion was continued for 8 to 12 hours after the cessation of uterine contractions. If this therapy was successful, these patients were given the original agent assigned for subsequent maintenance oral therapy.

Patients with subsequent rupture of membranes, cervical dilatation >5 cm, or clinical evidence of chorioamnionitis or oligohydramnios were considered to be study failures. Patients with recurrent preterm labor were given intravenous ritodrine or increased oral indomethacin therapy according to their original assignment.

A review of nursing notes and a patient interview by one of the authors approximately 48 hours after delivery were used to assess drug-induced side effects. Mothers were asked if they would be willing to use their assigned tocolytic agents in future pregnancies as a gauge of patient acceptance. Unpaired *t*-tests and χ^2 test statistics were used to compare the data from the two treatment groups. Statistical significance was defined as a p value of <0.05.

Results

The two treatment groups were similar, except that the indomethacin group had a significantly higher number of elective pregnancy terminations (Table I). There were no statistically significant differences in the two groups with respect to maternal outcome (Table II).

A significant difference was observed in maternal symptoms (Table III). Whereas only one patient in the ritodrine group had intravenous therapy discontinued as a result of maternal intolerance, a large percentage of patients voiced complaints of side effects such as palpitations, jitteriness, lightheadedness, headache, and dyspnea. The incidence of gastrointestinal symptoms was similar in both groups.

Four-quadrant amniotic fluid index values remained constant in the study population that received indomethacin tocolysis. Weekly amniotic fluid estimations were performed for up to 63 days of tocolytic ther-

Table I. Patient characteristics after randomization

Patient characteristic	*Ritodrine (n = 18)*	*Indomethacin (n = 22)*	*p Value*
Mean maternal age (yr)	20 ± 6*	23 ± 10	NS
Race			
White (%)	11	23	NS
Black (%)	89	73	NS
Hispanic (%)	0	5	NS
Nulliparous women (%)	56	45	NS
Previous preterm births (No.)	6	6	NS
Previous term births (No.)	7	20	NS
Abortions (No.)	7	26	<0.05
Mean gestational age at admission (wk)	29.9 ± 6.2	29.8 ± 4.9	NS
Mean initial cervical dilatation (cm)	1.8 ± 1.7	1.9 ± 1.3	NS
Mean initial cervical effacement (%)	61 ± 35	60 ± 39	NS
Mean frequency of initial contractions			
Every 1-5 min (%)	83	77	NS
Every 5-10 min (%)	17	23	NS
Twin gestations (%)	11	14	NS
Positive cervical cultures (%)	28	32	NS

*Mean and 95% confidence interval.

Table II. Maternal outcome

Variable	*Ritodrine (n = 18)*	*Indomethacin (n = 22)*	*p Value*
Mean interval to delivery (days)	27.9 ± 44.9*	25.5 ± 39.1	NS
Mean gestational age at delivery (wk)	33.8 ± 7.1	35.5 ± 5.7	NS
Attained 35th wk of gestation (%)	39	41	NS
Delivery delayed >48 hr (%)	83	91	NS
Delivery delayed >7 days (%)	67	68	NS
Initial total time in labor and delivery (hr)	14.7 ± 8.1	16.7 ± 11.9	NS
Antepartum days in hospital	3.2 ± 2.3	3.3 ± 1.9	NS
Required magnesium sulfate (%)	33	27	NS
Mean time on magnesium sulfate (hr)	17.8 ± 21.4	17.2 ± 22.5	NS
Initial study failures (%)	17	14	NS
Required maximum dosage (%)	28	27	NS
Subsequent PROM (%)	17	23	NS
Readmissions with PTL or PROM (%)	39	59	NS
Delivery route			
Vaginal (%)	78	68	NS
Cesarean section (%)	22	32	NS
Estimated blood loss at delivery (ml)	512 ± 339	615 ± 567	NS

PROM, Premature rupture of membranes; *PTL,* preterm labor.
*Mean and 95% confidence interval.

apy and failed to demonstrate a significant linear relationship between amniotic fluid volume and days of indomethacin administration (correlation coefficient = 0.17). Oligohydramnios was observed in a single fetus of a diamniotic twin gestation treated at 32 weeks. This infant's amniotic fluid index dropped for 10 to 2 cm during a 16-day course of indomethacin therapy. The other twin's amniotic fluid index values ranged from 7 to 10 cm.

The neonatal outcomes are presented in Table IV. There were no statistically significant differences in the two study groups.

Three neonates in the indomethacin group, two of whom were twins with discordant oligohydramnios, had clinical evidence of primary pulmonary hypertension shortly after birth. Serial ultrasonography had revealed lagging interval growth in both twins and oligohydramnios in twin B, but biophysical profiles were otherwise normal. Estimated fetal weights of both at 35 weeks of gestation were approximately 1500 gm. Oligohydramnios in twin B persisted 72 hours after discontinuation of indomethacin, and labor was induced for fetal indications. These twins were delivered by primary cesarean section after a second-stage arrest of labor and late decelerations in twin B (the infant with oligohydramnios). Twin A, a 1590 gm female, had an umbilical artery pH of 7.26. Twin B, a 1540 gm female, had an umbilical artery pH of 7.25. Each had 5-minute Apgar scores of 9 and each fell below the 10th percentile for weight at 35 weeks of gestation. Intubation in the delivery room was necessary in both twins and both began to have signs of pulmonary hypertension shortly

Table III. Patient symptoms

	Ritodrine (n = 18)	*Indomethacin (n = 22)*	*p Value*
Maternal intolerance (%)	6	0	NS
Maximum maternal heart rate (beats/min)	133 ± 22*	86 ± 12	<0.001
Minimum maternal systolic blood pressure (mm Hg)	99 ± 13	101 ± 15	NS
Minimum maternal diastolic blood pressure (mm Hg)	50 ± 11	57 ± 12	<0.001
Maximum fetal heart rate (beats/min)	163 ± 14†	145 ± 14‡	<0.001
Maternal symptoms			
Palpitations (%)	83	0	<0.001
Lightheadedness (%)	61	7	<0.001
Jitteriness (%)	83	5	<0.001
Dyspnea (%)	39	0	<0.01
Headache (%)	50	5	<0.001
Gastrointestinal symptoms (%)	44	54	NS
Positive patient acceptance (%)	61	95	<0.01

*Mean and 95% confidence interval.

†n = 20 to account for two sets of twins.

‡n = 25 to account for three sets of twins.

Table IV. Summary of neonatal outcome parameters

Neonatal outcome	*Ritodrine (n = 20)*	*Indomethacin (n = 25)*	*p Value*
Birth weight (gm)	2189 ± 1104*	2090 ± 1202	NS
5 min Apgar score	8.2 ± 2.3	7.8 ± 2.3	NS
Umbilical artery pH	7.31 ± 0.16†	7.30 ± 0.12‡	NS
No. of days in neonatal intensive care unit	11.1 ± 18.7	8.4 ± 21.4	NS
No. of days on ventilator	8.9 ± 27.3	3.0 ± 8.3	NS
Primary pulmonary hypertension (%)	0	12	NS
Patent ductus arteriosus (%)	0	8	NS
Intraventricular hemorrhage (%)	10	12	NS
Neonatal deaths (%)	5	4	NS

*Mean and 95% confidence interval.

†n = 14.

‡n = 19.

after admission to the neonatal intensive care unit. Twin A responded to hyperventilation therapy and was extubated on the second day of life without significant sequelae. However, twin B had a stormy neonatal course and required hyperventilation, tolazoline, and dopamine during 18 days of mechanical ventilation in the intensive care unit. Twin B was also treated with digitalization for a cardiomyopathy. Both twins were doing well at discharge.

The other patient whose infant had primary pulmonary hypertension presented in the twenty-fifth week of gestation and received indomethacin for 24 days before readmission with premature rupture of membranes. Amniotic fluid volume had been normal on three posttherapy ultrasonographic evaluations. The last dose of indomethacin was given approximately 8 hours before delivery of a 1240 gm female infant with a 5-minute Apgar score of 9 and an umbilical artery pH of 7.41. This neonate required immediate intubation and responded to hyperventilation for clinically apparent primary pulmonary hypertension. She subsequently required mechanical ventilation for 14 days and eventually needed indomethacin for closure of a patent ductus arteriosus.

Comment

This is the first randomized trial that compares long-term therapy with indomethacin and β-sympathomimetics. Our data suggest that these tocolytic drugs are comparable in delaying preterm delivery.

This study did not address the wisdom of tocolytic use in the treatment of preterm labor, although this has been questioned.[11] Because ritodrine is approved by the Food and Drug Administration for this indication and is widely used, we used it as the standard with which to compare indomethacin.

Morales et al.[3] recently reported a randomized trial

that compared short-term indomethacin with ritodrine for primary therapy in 106 preterm pregnancies. All undelivered patients were subsequently given oral β-sympathomimetic maintenance therapy until delivery. In that study delay of delivery for >48 hours was comparable in the two treatment groups, and no significant difference in gestational age at delivery or birth weight was reported. In addition, no significant differences in neonatal outcome were observed. A significant difference in maternal side effects was observed; 24% of the patients discontinued β-sympathomimetic therapy as a result of maternal intolerance. In the study by Morales et al., as in ours, no significant maternal side effects from indomethacin were reported. Drup-induced oligohydramnios was observed in 11% of the indomethacin group, which prompted discontinuation of therapy. A large cost differential between intravenous ritodrine therapy and short-term oral indomethacin therapy was also reported ($560 vs $33).

Our data on the difference in maternal side effects between these two tocolytic agents are similar to those of previous studies. Most patients that receive indomethacin are asymptomatic or have minor nausea or heartburn.[1, 10] Some patients receiving intravenous ritodrine report significant maternal cardiovascular and central nervous system side effects.[12]

Neonatal adverse effects have been reported previously with indomethacin therapy. Transient constriction of the ductus arteriosus has been reported,[6] which may be more common in fetuses of later gestation age. Case reports have suggested an association of indomethacin treatment with primary pulmonary hypertension.[7] However, large clinical experiences with short-term indomethacin therapy have failed to document such neonatal complications in a total of 584 exposed fetuses[1, 3, 8-10] who received the drug for ≤48 hours. However, Gerson et al.[13] observed two cases with complications among 31 fetuses exposed to long-term indomethacin therapy. Grella and Zanor[14] reported primary pulmonary hypertension in 4 of 29 infants, and Rubaltelli et al.[15] found five cases of pulmonary hypertension in 29 infants, all of whom were exposed to a 5-day course of indomethacin therapy. In our study the pulmonary hypertension in both fetuses of a twin pregnancy may have been attributable to long-term indomethacin therapy, but growth retardation is a confounding factor. The primary pulmonary hypertension in the other pregnancy is otherwise unexplained. In spite of these complications, all three neonates who experienced pulmonary hypertension are now alive without sequelae.

Recently, a number of clinical reports of indomethacin-induced oligohydramnios have appeared in the literature.[16, 17] In addition, indomethacin has been shown to reduce amniotic fluid volumes in pregnancies that are complicated by severe polyhydramnios.[18] This effect appears reversible with discontinuation of therapy and is probably a result of the ability of indomethacin to decrease fetal urine output.[17] The majority of the patients in our study who received long-term indomethacin therapy did not have oligohydramnios. The one case of nonreversible oligohydramnios observed in a complicated twin gestation was probably the result of growth retardation. Oligohydramnios is dose dependent[16] and occurs more frequently at a dosage of 200 mg of indomethacin per day but not at 100 mg per day, which most of our patients received.

We conclude that indomethacin is as effective as the β-sympathomimetics for tocolysis, although this study was discontinued before the numbers reached a level necessary to minimize type II error. We had not previously seen primary pulmonary hypertension when we limited indomethacin therapy to 24 to 48 hours. Thus we speculate that if indomethacin treatment is prolonged for >48 hours, there may be an increased risk of this complication.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. THOMAS H. KIRSCHBAUM, Los Angeles, California. This article extends a related study published in 1980.[1] In the earlier work, which was a randomized blind study, the efficacy of indomethacin in inhibiting preterm labor was compared in two groups of patients: those treated with indomethacin and those who did not receive indomethacin. The study was not placebo controlled in a rigorous sense, because 30% of the women in the control group ultimately received other tocolytic drugs. Entry criteria were virtually the same as in this study. Treatment failure was defined as a progressive change in the cervix that occurred >2 hours after initial treatment or the attainment of >4 cm of cervical dilatation. Fifteen patients were studied in each group, and there was a statistically significantly larger incidence of treatment failure in the control group as opposed to drug-treated patients at 24 and 48 hours after the initiation of therapy. No effect on outcome as measured by life-threatening newborn complications or perinatal death was seen, although the numbers of cases were relatively small. Mean values indicate that the drug-treated group averaged 3.2 weeks of prolongation of pregnancy after treatment, whereas the control group averaged 3.0 weeks of prolongation.

In this article the authors make two points. The first is that indomethacin is less expensive than ritodrine, has fewer maternal side effects, and is as effective as ritodrine, as the outcome measurements indicate. The second point is that pulmonary hypertension was seen in two of the pregnancies and in three of the infants of 22 women who received indomethacin, whereas none of the 18 women treated with ritodrine experienced those neonatal complications. There are, I believe, some questions that concern the validity of those conclusions, and I should like to pose two additional questions.

The differences in the reproductive histories of the two study groups are regrettable because they constitute uncontrolled variables. Eighteen of the ritodrine-treated women had 20 prior pregnancies, which included seven abortions; 22 of the women treated with indomethacin had 52 prior pregnancies, which included 26 abortions. The interval to delivery and hence of drug therapy for both groups shows a great deal of scatter from the respective means. I calculate a mean and SD of 27.9 ± 22.9 days for ritodrine and 25.5 ± 20 days for indomethacin. Whether the unpaired *t* test might not be misleading for differences between means when frequency distributions are not in a gaussian curve is a serious question. Also, these results were obtained with the use of magnesium sulfate in about 30% of both groups after the initial drug failed to control labor. This means that a comparison of the effects of indomethacin and ritodrine alone cannot be made from these data. In fact, the data suggest bimodal or multimodal frequency distributions. This problem is further exhibited in the authors' Table IV, in which the 8.9 average number of days on the ventilator for the infants born to women treated with ritodrine has a standard deviation of 13.9 days. This indicates a number of extreme outliers form the mean value. Again, the choice of the statistical analytic technique used here is questionable.

These factors do not help to prove a negative hypothesis—here, that there is no difference between these two forms of therapy. This premise differs from that of the article written in 1980, in which a positive hypothesis was pursued. A negative hypothesis can never be proved; it simply displays the experimental design, the number of cases studied, and the data analysis and makes the argument that if there were a difference, it would have been discerned. In this study all three of those elements are arguable. As the authors acknowledge, the number of cases studied is too small to minimize the chance of false-positive errors or β-errors. The presence of a difference between these two forms of therapy might have been seen if more cases had been studied or, for example, if a design that included no magnesium sulfate therapy had been used.

Would such a study have been ethically acceptable? I think so, if the work of others in this area is examined. The authors' 1980 article is one of two studies that purport to show the effects of indomethacin in inhibiting labor for at least 24 hours.[1, 2] Two other studies show that the effect of indomethacin tended to delay labor for 48 hours[2, 3] without effect on fetal and neonatal deaths (five studies[1-5]) or respiratory distress syndrome (four studies[1, 2, 4, 5]). β-Sympathomimetic tocolysis has been shown to be effective in delaying the delivery of infants in treated patients (as compared with control subjects) for 24 hours (five studies[6-10]) or for 48 hours (two studies[9, 10]) without affecting perinatal mortality (14 studies[6-10]) or respiratory distress syndrome (12 studies[7-10]). It is a recurring observation that data in this area fail to show an effect on morbidity or survival, and in the absence of that evidence of benefit, not treating patients would seem to be acceptable. The maintenance of pregnancy with oral β-sympathomimetic agents has not been shown to be of

value, and no data exist in regard to the effectiveness of oral maintenance doses of prostaglandin synthetase inhibitors.

A final question relates to the average time of nearly 1 month that elapsed between treatment and delivery in these patients. The question, of course, is whether these patients were ever truly in preterm labor. Entry criteria consist of uterine contractions that occur roughly every 5 to 7½ minutes, cervical change, or a cervix with dilatation >2 cm or effacement >75%. It is hard to know what constitutes 75% of normal effacement, particularly in multiparous women, and the term *change* allows subjective evaluation. All of us have seen patients who were clearly not in labor but who met the entry criteria. Until direct biochemical markers of labor appear, this question will continue to make good experimental design, large numbers of cases, and careful statistical evaluation essential.

The data allude to the effect of long-standing indomethacin treatment on ductus arteriosus stenosis and resultant pulmonary hypertension. Ductal patency is dependent on the production of large amounts of prostaglandins by the ductus endothelium and muscularis. Inhibition of uterine contractions with indomethacin in fetal animals and in human neonates has been demonstrated to constrict the ductus and to produce fetal pulmonary hypertension with possible neonatal consequences. Two of 22 treated with indomethacin constitute evidence that suggests that the risks of indomethacin treatment may be greater than the benefits. The use of indomethacin without clear evidence of benefit beyond the prolongation of pregnancy by 24 to 48 hours may be seriously questioned.

The authors have added new data that address this important area. Regrettably, they have not provided a great deal of clarification as a result of the intrinsic difficulty of research in which labor cannot rigorously and objectively be defined.

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DR. DAVID COTTON, Houston, Texas. I have some comments and then a question. As you know, Dr. Moise in our group has been very interested in the effects of indomethacin on the fetus. Because of his work that associates closure of the fetal ductus and oligohydramnios with indomethacin treatment of pregnant women, we have a rather cumbersome protocol for follow-up of these patients. In that protocol we evaluate ductal patency once a week and assess amniotic fluid volume twice a week.

We have seen the occurrence of fetal ductal closure and tricuspid insufficiency within about 48 hours of the initial administration of indomethacin, and we have also seen tricuspid insufficiency that did not resolve in 48 hours. In one case tricuspid insufficiency actually lasted 3 days but did finally resolve. Thus the adverse effects of indomethacin can sometimes be seen fairly quickly.

We have never been able to define the timing of amniotic fluid reduction. In some women a reduction in amniotic fluid volume develops after only 3 or 4 days of indomethacin therapy. Other patients will be treated with indomethacin for as long as 3 weeks before a decrease in amniotic fluid results. We still feel that it is very important to monitor amniotic fluid volume in patients, because we have had some evidence that neonatal renal failure may occur in women who have had prolonged oligohydramnios.

However, the side effects described in this study pertain to those in the β-sympathomimetic group that are usually seen very early on and that in many instances start to decrease over a 3-day to 7-day period if the patient becomes asymptomatic. Terbutaline may continue to have its effect of relaxation of the uterus.

Amniotic fluid must be measured on a regular basis via ultrasonography. The side effects of a medication may initially be more complicated to follow, but after an initial evaluation, sophisticated monitoring is not required, even if the ductus is not evaluated. Do you think these agents really are comparable in terms of how easy and how expensive they are for the patients to take them?

DR. ROBERT RESNIK, San Diego, California. Several years ago, Dr. Irwin Merkatz wrote a multiinstitutional collaborative study on the effects of ritodrine, which led to the approval of ritodrine by the Food and Drug Administration. As I recall (and he may wish to comment on the clarity of what I'm going to say), the mean

number of days gained before delivery in the control group was 21. In Dr. Niebyl's study the mean was 25 days in the indomethacin group and 28 days in the ritodrine group.

Would you comment on this, in light of Dr. Kirschbaum's previous comments, and define the onset of true labor to aid in determining the efficacy of these drugs?

Dr. Warren M. Crosby, Oklahoma City, Oklahoma. The neonatologist who treats primary pulmonary hypertension in which the ductus remains open is in a real bind, because once he or she closes the ductus, the pulmonary hypertension becomes worse. This study does not comment on the three babies that were treated in that manner. I would like to know if those babies became clinically better or worse.

Dr. Thomas J. Garite, Orange, California. We have just completed an analysis of some data and have found an interesting association between indomethacin and necrotizing enterocolitis. Did you see any cases of necrotizing enterocolitis in your group?

Dr. Niebyl (Closing). First of all, I'll try to answer Dr. Kirschbaum. His questions about the statistics are well taken, but I think that we used the appropriate tests for the number of patients. We had only 40 patients, and I think that applying a more sophisticated method might have gotten us into trouble.

The point about untreated controls is an important one that several people have reiterated. We had originally done a placebo-controlled trial with indomethacin, which showed that indomethacin treatment was effective, at least during that 48 hours of therapy. The important questions really concern the effectiveness of indomethacin in prolonging pregnancy or in making a significant difference in the health of the baby. I'm not sure that it does.

The reason we designed this trial, in which patients receive treatment with the drug for a month, was really to answer those questions. This study was simply a comparative study of the effects of ritodrine and indomethacin treatment. The study was not done to examine the whole issue of whether untreated control subjects do as well as drug-treated subjects.

I think that the answers may be somewhere in the middle; a certain subgroup of patients will benefit from treatment, but there are also certain patients who are undoubtedly not in preterm labor and who, if left untreated, would not be delivered of their infants. It's very difficult to identify those patients. We certainly know that patients who come in repeatedly for examinations and who do not experience cervical change are still at risk of preterm delivery. Thus there are some groups of patients who may benefit.

There's certainly a placebo effect if all of the placebo-controlled trials or untreated control trials are examined. There is a success rate of 20%, 30%, or sometimes even as high as 50% to 60% in avoiding preterm delivery without treatment. Our problem is the difficulty in diagnosing which patients are really in preterm labor.

Dr. Cotton mentioned the work on Doppler ultrasonography by the group at Baylor. I think that if I were designing this study to start today, I would incorporate some sort of Doppler follow-up with these babies, because as the Baylor study indicates the constriction can happen very quickly. We did monitor amniotic fluid volume. We did not look at the ductus. Dr. Cotton's question really related more to which medication is the more difficult to use. I think his point that ritodrine is more difficult to use initially is well taken. Tachycardia or other problems may result immediately with ritodrine treatment, but tolerance to the medication may develop in a patient with long-term therapy. I feel that trouble with indomethacin may occur in long-term therapy, as it did in the few cases that we reported.

Dr. Crosby asked me whether infants who had primary pulmonary hypertension in which the ductus remained open improved after closure of the ductus. All those babies responded very well, did well in the nursery, and went home without problems. They were sick initially, but they did respond to therapy and did not have any significant long-term problems.

Dr. Garite asked about necrotizing enterocolitis. We didn't see any, but the association has been reported in the literature. However, this type of enterocolitis does not occur often, and there has been no significant association of the disease with indomethacin treatment.

The induction of premature luteolysis in normal women—follicular phase luteinizing hormone secretion and corpus luteum function in the subsequent cycle

Michael R. Soules, MD,[a] William J. Bremner, MD, PhD,[b, c] Kristine D. Dahl, PhD,[b, c] Jean E. Rivier, PhD,[d] Wylie W. Vale, PhD,[d] and Donald K. Clifton, PhD[a]

Seattle, Washington, and La Jolla, California

Women with luteal phase deficiency have been shown to have an increased frequency of luteinizing hormone pulses in the early follicular phase of the menstrual cycle. Because progesterone is known to modulate luteinizing hormone secretion, it has been hypothesized that the decreased progesterone secretion in a previous luteal phase deficiency cycle could lead to the abnormal luteinizing hormone secretory pattern in the ensuing early follicular phase. With the possibility that the higher luteinizing hormone pulse frequency might lead to another deficient luteal phase, it becomes conceivable that luteal phase deficiency could be self-perpetuating. To test this hypothesis, luteal phase deficiency was induced in six normal women by decreasing luteinizing hormone support of the corpus luteum with a gonadotropin-releasing hormone antagonist Nal-Glu, administered twice daily beginning in the midluteal phase after a control cycle. During the antagonist-treated luteal phase, each subject met the predetermined criteria for induced luteal phase deficiency: a 33% or greater decrease in integrated progesterone from the control cycle and an integrated progesterone level <100 ng/ml per day. Luteinizing hormone secretion patterns were determined by frequent blood sampling performed every 10 minutes for 12 hours in the early follicular phase of the control cycle and the cycle after antagonist administration. Daily luteal progesterone levels were measured in the control, treatment, and posttreatment cycles. Each volunteer served as her own control. Standard parameters were compared between the control and posttreatment pulse studies in the early follicular phase: (1) luteinizing hormone pulse frequency was 9.5 ± 1.0 vs 10.0 ± 0.9 pulses/12 hours, control vs posttreatment, respectively, $p = 0.5$; (2) luteinizing hormone pulse amplitude was 11.0 ± 1.3 vs 12.0 ± 2.2 ng/ml, $p = 0.6$; and (3) luteinizing hormone mean level was 19.4 ± 2.3 vs 22.2 ± 3.3 ng/ml, $p = 0.1$. Corpus luteum function was also compared between the control and posttreatment cycles. Luteal phase length was 13.7 ± 0.6 vs 12.7 ± 0.6 days, $p = 0.08$. Integrated progesterone values were 136.9 ± 12.9 vs 130.5 ± 11.3 ng/ml per day, $p = 0.5$. Therefore no discernible abnormalities in early follicular luteinizing hormone secretions or corpus luteum secretion of progesterone occurred after an induced luteal phase deficiency cycle. We conclude that luteal phase deficiency is not self-perpetuating and that the rapid luteinizing hormone secretion pattern found in the early follicular phase in women with luteal phase deficiency is not a result of decreased progesterone levels in the preceding luteal phase, but rather appears to be a result of dysfunctional suprahypothalamic neuroendocrine modulation of the gonadotropin-releasing hormone pulse generator. (Am J Obstet Gynecol 1991;164:989-96.)

Key words: Luteal phase deficiency, progesterone, luteolysis, gonadotropin-releasing hormone antagonist, gonadotropin secretion, corpus luteum

From the Departments of Obstetrics and Gynecology[a] and Medicine,[b] University of Washington, Veterans Administration Medical Center,[c] and the Peptide Biology Laboratory, The Salk Institute.[d]
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W.W.V. is a senior Clayton Foundation investigator.
Reprint requests: Michael R. Soules, MD, Department of Obstetrics and Gynecology, RH-20, University of Washington, Seattle, WA 98195.
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There is a complex relationship between the hypothalamic-pituitary (neuroendocrine) unit and the corpus luteum in women. A well-described shift in luteinizing hormone (LH) pulse frequency and amplitude occurs from the follicular to the luteal phase, with fewer LH pulses of higher amplitude present in the second half of the cycle. This slowing of the LH secretion pattern in the luteal phase is a result of progesterone feedback on the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator, which is mediated by endogenous opioid peptides.[1, 2] Progesterone secretion by the corpus luteum is pulsatile as well and has been shown to be coupled with LH pulses in the midluteal

and late luteal phases.[3-5] LH appears to be required in both a permissive and a quantitative manner for normal progesterone secretion by the corpus luteum.[3, 6] Because the follicular phase LH pulse pattern is of relatively high frequency and low amplitude, there is a necessary increase in the LH secretory rate that accompanies the death of the corpus luteum. Withdrawal of progesterone is the presumptive stimulus for the luteal to follicular increase in gonadotropin pulse frequency.

Luteal phase deficiency is a well-described aberration of the menstrual cycle that usually consists of decreased progesterone secretion by the corpus luteum. Luteal phase deficiency can be a phenomenon that occurs sporadically in normal women, without clinical significance, or it can be the cause of infertility or habitual abortion.[7] We have reported significant abnormalities in the hypothalamic-pituitary-ovarian axis in women with luteal phase deficiency. They have an increased LH pulse frequency in the early follicular phase, decreased mean LH levels during the LH surge, decreased bioactive LH levels in the midluteal and late luteal phases, and decreased progesterone pulse amplitude in the luteal phase, accounting for their low integrated progesterone levels.[8, 9] In a separate study we were able to produce luteal phase deficiency in normal women by inducing a supraphysiologic LH pulse frequency during the follicular phase.[10] On the basis of these studies, we consider the rapid early follicular LH pulse pattern a key element in the pathophysiology of luteal phase deficiency and believe it is the probable cause of the abnormal LH and progesterone secretion later in the cycle.

Thus with our focus on the rapid early follicular LH pulse pattern as a key element in the pathogenesis of luteal phase deficiency and the ability of progesterone to modulate the LH pulse frequency, we hypothesized that luteal phase deficiency could be self-perpetuating. The following hypothesis was entertained: decreased progesterone secretion in a given luteal phase could result in an excessively rapid gonadotropin pulse pattern in the subsequent follicular phase which, in turn, would lead to luteal phase deficiency in that and future cycles. This study was designed to test this hypothesis. To this end, luteal phase deficiency (premature luteolysis with decreased luteal progesterone levels) was induced in normal women with a GnRH antagonist and the subsequent cycle was monitored in terms of the follicular LH secretion pattern and corpus luteum function.

Methods

Subjects. This study was designed for normal volunteers to serve as their own controls. These women ($n = 6$) were between 26 to 33 years of age, within ±10% of their ideal body weight (Metropolitan Life Insurance Co. tables, 1980), and no more than moderate exercisers. They had a history of 27- to 32-day menstrual cycles, at least one normal biphasic basal body temperature chart, and met the following hormonal criteria in the midluteal phase of a menstrual cycle preceding the study: progesterone ≥12 ng/ml, prolactin <20 ng/ml, and testosterone ≤40 ng/dl. The protocol was approved by the Human Subjects Committee of the University of Washington and informed consent was obtained from each woman.

There was a preset criterion for inclusion in the study that the GnRH antagonist administration induce a luteolysis sufficiently similar in terms of the pattern and time course of decreased progesterone levels to that seen in women with spontaneous luteal phase deficiency as previously described.[8] To this end, we required each subject to have a luteal phase of 13 or more days and an integrated progesterone level >100 ng/ml per day in the control cycle. In the luteal phase of the treated cycle they had to have an integrated progesterone level <100 ng/ml per day that was also a minimum of 33% below the integrated progesterone in their control cycle. Whereas a more profound and dramatic luteolysis could have been achieved with the Nal-Glu GnRH antagonist, depending on the dose, the decreased progesterone levels observed in spontaneous luteal phase deficiency cycles tend to be modest (around 33%) and usually occur in the latter half of the luteal phase. Some women with luteal phase deficiency had entirely normal levels of progesterone until the midluteal phase.[8] Fifteen subjects volunteered for the study and six met the criteria. Of the subjects who did not meet the study criteria, five had low integrated progesterone levels in the control cycle and the other four had insufficient luteolysis.

Protocol. The study encompassed three menstrual cycles. The protocol is summarized in Fig. 1. Daily blood samples were obtained from cycle day 10 until the onset of the next menstrual period in all three cycles. These samples were allowed to clot, serum was separated, aliquoted, and frozen at −4° C until assayed for LH and progesterone. A 12-hour admission to the Clinical Research Center occurred in the early follicular phase (cycle days 1 to 4) of the second and third study cycles. During these admissions (8 AM to 8 PM) a heparinized intravenous line was placed, through which 5 ml venous blood samples were drawn every 10 minutes (total, 73 samples). The serum from these samples was frozen at −4° C and subsequently assayed for LH.

The Nal-Glu antagonist injections were initiated in the midluteal phase of study cycle two and were administered subcutaneously every 12 hours. The injections began on cycle days +5 or +6 in relation to the LH surge (day 0) and were continued until the onset of the next menstrual period. The dose of the Nal-Glu

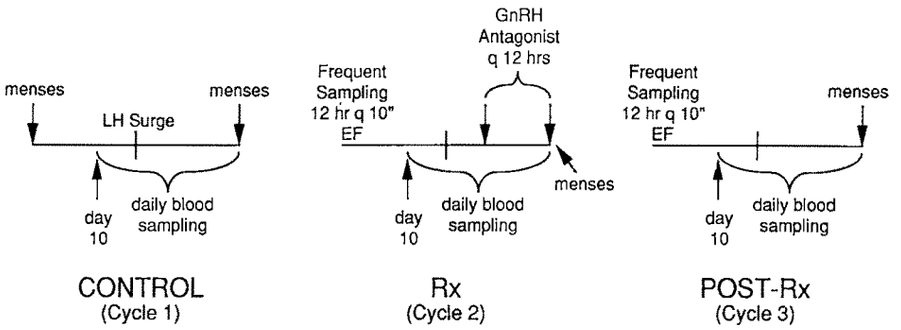


Fig. 1. The study protocol encompassed three menstrual cycles. The luteal phase of cycle one and the early follicular *(EF)* phase of cycle two were control intervals for luteal progesterone and follicular LH secretion, respectively. Luteal phase deficiency was induced with a GnRH antagonist administered from the midluteal phase of cycle two until the subject's next menstrual period (*Rx*, treatment). LH and progesterone secretion were monitored in cycle three.

antagonist was determined on the basis of our previous experience with its administration in the luteal phase[11] and was either 2.5 μg/kg or 5.0 μg/kg for each injection. The daily blood sampling increased to every 12 hours during antagonist administration, which varied between 5 and 8 days in duration.

Assays. Serum LH was measured by double antibody radioimmunoassay as previously described[12] with reagents supplied by the National Institutes of Health with LER 907 as the reference preparation. The sensitivity of the LH assay was 6 ng/ml, with intrassay and interassay coefficients of variation of 5.5% and 8.4%, respectively.

Serum progesterone was measured by radioimmunoassay with reagents supplied by Diagnostic Products Corp. (Los Angeles). This assay had a cross-reactivity of 0.3% with 17-hydroxyprogesterone and <0.01% with testosterone, estradiol, pregnenolone, and cortisol. The assay sensitivity was 0.5 ng/ml, and intraassay and interassay coefficients of variation were 9% and 12%, respectively.

Statistics. Luteal phase length in this study was defined as the number of days that occurred subsequent to the LH surge (peak LH value, day 0) and before the next menstrual period. Integrated progesterone values were calculated for each subject's luteal phase by adding the daily serum progesterone levels together beginning with the day of the LH surge; they are expressed as nanograms per milliliter per day.

For each subject there were 73 LH data points from the early follicular phase admissions in study cycles two and three. The following pulse parameters were calculated for LH: frequency, amplitude, and mean level. An adaptive threshold method (DC3) was used to determine the frequency and amplitude of pulses as previously described.[13]

Each volunteer served as her own control. Comparisons were made between study cycles with the paired Student t test for luteal length, integrated progesterone, and pulse parameters. The data are presented as mean ± SEM unless otherwise indicated. A p value ≤ 0.05 was considered significant.

Results

A decrease in serum progesterone levels in cycle two that simulated luteal phase deficiency was a requirement of this study. Thus mean integrated progesterone levels over the luteal phase decreased from 136.9 ± 12.9 to 80.7 ± 7.2 ng/ml per day between the control and treatment cycles (Fig. 2). Not unexpectedly, mean luteal phase length also decreased from 13.7 ± 0.6 to 11.2 ± 0.8 days ($p < 0.01$) between the control and treated cycles (Fig. 2). Therefore a luteal phase deficiency–like condition was successfully induced in these normal women.

The posttreatment cycle was carefully examined to ascertain whether this luteal phase deficiency state had a carry-over effect(s) into the subsequent cycle. The LH pulse pattern in the early follicular phase of the posttreatment cycle was compared with the pattern before treatment. A representative pair of early follicular LH secretion patterns from a particular volunteer are illustrated in Fig. 3. Both patterns appear to represent normal early follicular LH secretion. In fact, when the LH secretory parameters were compared for the entire group, there were no differences in frequency, amplitude, or mean level between the control and posttreatment sampling intervals (Table I).

In terms of luteal phase length and serum progesterone levels, the posttreatment cycle was similar to the control cycle. There was a tendency toward a decreased mean luteal phase length (12.7 ± 0.6 vs 13.7 ± 0.6 days, respectively, $p = 0.08$, Fig. 2). However, the mean integrated serum progesterone levels were nearly iden-

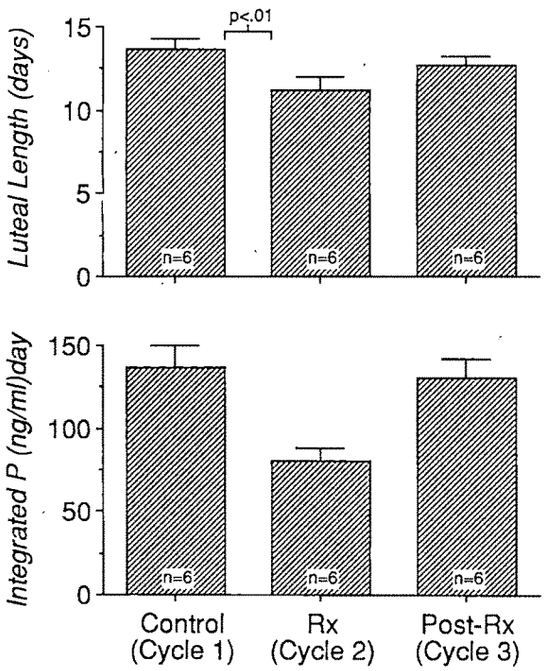


Fig. 2. Corpus luteum function in each of the three cycles is illustrated. Luteal phase deficiency was successfully induced in the treatment *(Rx)* cycle.

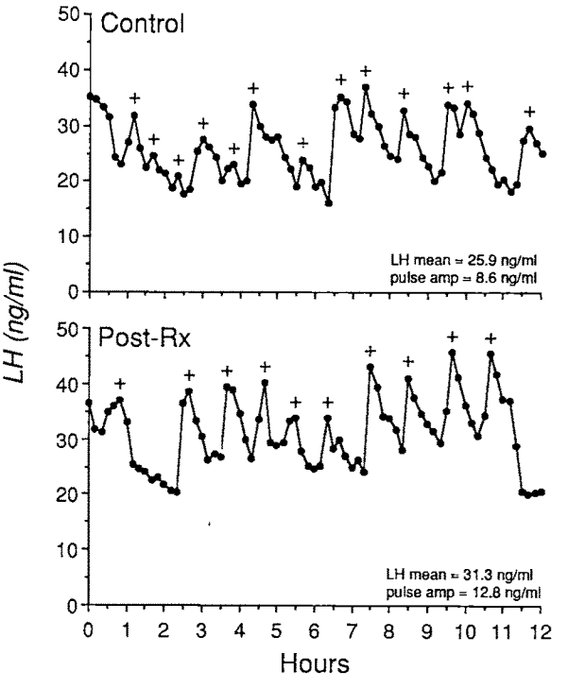


Fig. 3. The LH secretory pattern over 12 hours is illustrated for subject B in the early follicular phase of the control and posttreatment cycles. Secretory episodes *(pulses)* are indicated with a *plus sign.*

tical at 130.5 ± 11.3 vs 136.9 ± 12.9 ng/ml per day, $p = 0.5$ (Fig. 2). Therefore there was no evidence of a luteal phase deficiency–like condition in the posttreatment cycle.

Comment

Luteal phase deficiency is both a controversial and a diverse disorder with a number of investigators debating whether it actually occurs. It certainly does exist because it has been well documented in many different settings: post menarchal[14]; premenopausal[15]; post partum[16]; with strenuous exercise[17]; with dieting[18]; and in "normal" women who volunteer as control subjects.[19,20] It can be induced by interfering with folliculogenesis,[21] luteal phase luteinizing hormone suppression,[22] or a supraphysiologic early follicular LH pulse frequency.[10] Therefore the relevant question is not whether it exists, but does it occur regularly in some women and thereby cause infertility or recurrent abortions? The latter question remains controversial, primarily because there is not a simple effective test to determine whether luteal phase deficiency is present in the clinical setting.[23] It is our considered opinion that luteal phase deficiency is a primary cause of infertility and habitual abortion in 5% to 10% of a population referred for subspecialty care.

Luteal phase deficiency consists of either decreased corpus luteum function (inadequate secretion of progesterone, estradiol, and inhibin) or an insufficient hormonal effect at the level of the endometrium. Whereas considerable research attention has been directed at insufficient end-organ response in luteal phase deficiency, very little consistent evidence has come forth.[23] Therefore in both the research and clinical setting, luteal phase deficiency as we understand it today can primarily be characterized as inadequate hormonal secretion. Many reasons for inadequate hormonal secretion by the corpus luteum have been proposed: (1) inadequate follicular development, (2) a dysfunctional LH surge, (3) insufficient LH support of the corpus luteum in the luteal phase, (4) endogenous corpus luteum defect(s) that preclude adequate function, and (5) premature or excessive luteolysis.

There has been a relative paucity of studies that have investigated the pathogenesis of spontaneous luteal phase deficiency in women, especially with regard to endogenous defects in the corpus luteum itself. There are good indications that interference with folliculogenesis can lead to luteal phase deficiency[21] and abnormal follicular phase follicle-stimulating hormone (FSH) levels have been reported in luteal phase deficiency.[19,20,24] However, we were not able to find any abnormalities in FSH levels, follicle size, or estradiol levels in an in-depth study of 10 women with sponta-

Table I. LH secretory parameters in the early follicular phase

Cycle	*Subject*	*LH pulses (No. per 12 hr)**	*LH pulse amplitude (ng/ml)†*	*Mean LH (ng/ml)‡*
Control				
	A	9	12.9	25.3
	B	13	8.7	26.0
	C	6	14.8	20.4
	D	10	5.8	11.4
	E	8	11.0	15.1
	F	11	12.6	18.5
	Mean ± SEM	9.5 ± 1.0	11.0 ± 1.3	19.4 ± 2.3
Posttreatment				
	A	10	16.1	31.3
	B	10	12.8	31.3
	C	6	20.1	24.9
	D	12	8.2	15.9
	E	10	7.2	13.7
	F	12	7.3	16.0
	Mean ± SEM	10.0 ± 0.9	12.0 ± 2.2	22.2 ± 3.3

*$p = 0.5$.
†$p = 0.6$.
‡$p = 0.1$.

neous luteal phase deficiency.[8] That study did find deficiencies in the LH surge and in the LH support of the corpus luteum. We believe this lack of hormonal stimulus to the corpus luteum is a major component of the pathophysiology of luteal phase deficiency. In addition, two further pieces of evidence led us to focus on the neuroendocrine component (GnRH pulse generator) as the basis for luteal phase deficiency in most women with this condition. First, we have quite consistently found a significant increase in LH pulse frequency (with the expected decrease in LH pulse amplitude) in the early follicular phase of luteal phase deficiency cycles.[9] Other investigators have found abnormalities (both rapid and slow) in early follicular LH pulse frequency in luteal phase deficiency as well.[25, 26] Second, we were able to induce significantly decreased luteal phase levels of progesterone and bioactive LH in normal women by means of a supraphysiologic LH pulse frequency in the follicular phase.[10] Therefore it appeared to us that investigating the altered GnRH pulse generator in the follicular phase might be the key to understanding the pathophysiology of luteal phase deficiency.

One possible cause of the increased secretory rate of LH in luteal phase deficiency could be the low progesterone levels in the prior luteal phase. Progesterone is known to modulate (slow) the GnRH pulse generator,[1] thus too little progesterone could lead to a premature and excessive increase in LH secretion and, potentially, luteal phase deficiency in the next cycle. This study found no compelling evidence that luteal phase deficiency perpetuates itself into subsequent cycles. The lack of decreased progesterone secretion was to be expected after induced luteal phase deficiency when no changes in the LH secretory pattern in the early follicular phase occurred. It now becomes necessary to look at other modulators of the GnRH pulse generator to understand the alterations in early follicular LH secretion and the pathophysiology of luteal phase deficiency. There are many examples of suprahypothalamic influences on the GnRH pulse generator that have profound effects on the menstrual cycle. Conditions such as anorexia nervosa,[27] hypothalamic amenorrhea,[28] heavy exercise,[29] and dieting[30] all have in common either psychological or physical stress, abnormal gonadotropin secretion patterns, and menstrual dysfunction. We postulate that luteal phase deficiency as a mild and subtle form of menstrual dysfunction commonly occurs because of stress-induced alterations in the GnRH pulse generator, mediated by neurotransmitters.

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Discussion

Dr. Isaac Schiff, Boston, Massachusetts. Dr. Soules is clearly a leader in the field of luteal phase deficiency. His solid and important research has furthered our knowledge of the subject since Dr. Georgeanna Seegar Jones first described the condition over 40 years ago. Luteal phase deficiency was defined as the unusual condition in which the endometrium was greater than 2 days out of phase as documented by endometrial sampling. The treatment that was suggested was progesterone supplementation. Numerous studies that indicate the beneficial results of progesterone therapy for luteal phase deficiency, when traditionally diagnosed with a biopsy specimen, constitute provocative evidence for this disorder being a cause of reproductive failure.

I read Dr. Soules' article with great interest and in the brief time allowed me here I would like to formulate my comments on it in a number of questions—it being true that every scientific article of significance raises as many questions as it answers.

His protocol asked whether normal women can withstand an assault on their hypothalamic pituitary gonadal axis by internally adapting and resetting the pulse generator. The answer to the posed question was clearly no. There was no evidence of induction of a luteal phase deficiency–like condition. Perhaps some persons are more susceptible to hypothalamic pituitary gonadal axis perturbations than are others. Is it true that most persons with some stress or external stimuli can adapt and reorganize the hypothalamic pituitary gonadal axis

for the next menstrual cycle and therefore resist the tendency of luteal phase deficiency to develop? Is it true that others cannot because of some defect of the hypothalamic pituitary gonadal axis that, although subtle, is serious enough to produce luteal phase deficiency? One could thus obtain different data if more patients were studied or if an infertile group of women were so examined. After all, the authors state that luteal phase deficiency can be a phenomenon that occurs sporadically in normal women. On this same matter, how long do the effects of alteration in progesterone persist to control gonadotropin secretion?

In his article, Dr. Soules stresses the LH pulsations as an explanation for luteal phase deficiency. Given the unfair advantage of hindsight and being cognizant of the negative results, one might ask whether there is too much emphasis on LH. According to the work of Hutchison, the corpus luteum can be maintained with infrequent pulses of GnRH (hence LH) during the luteal phase, but its usual duration cannot be extended with physiologic doses. And indeed other factors such as prostaglandins may be operative. High levels of LH in the follicular phase may induce multiple folliculogenesis, and the increase in estrogens from these follicles could produce premature luteinization and the corpus luteum defect. Could, then, the addition of LH in the luteal phase prevent luteal phase deficiency? Finally, if LH is that critical then it implies that the hypothalamus pituitary-luteal axis is homeostatic, as are other pituitary target organs. I think that this is not the case, and it is a good thing too, because then the pituitary would always be trying to rescue the corpus luteum at the end of each nonconception cycle.

Some authors believe that luteal phase deficiency might be induced by an alteration in FSH levels in the follicular phase. Dr. Soules, did you happen to measure FSH, and if so, did you find a difference?

In the day-to-day clinical situation, many physicians do not have access to Dr. Soules' laboratory expertise, and they are compelled to rely on results of endometrial biopsies. Given their well-known drawbacks, I wonder whether in carrying out his study Dr. Soules performed any biopsies?

To induce experimentally the condition of corpus luteum defect, the investigators used a GnRH antagonist. The procedure, as the authors acknowledge, shortens the luteal phase. The short luteal phase might itself be only one type of corpus luteum defect. A further reaching study design would have called for a luteal phase of 12 to 14 days' duration and an integrated progesterone level that is decreased. I welcome Dr. Soules' suggestions on this point.

Perhaps there is another conceptual framework for the model of the self-perpetuating nature of luteal phase deficiency. If we assume that the following statements are more or less basic observations: (1) progesterone in nonconception cycles declines at some point and (2) the pulse generator does not count the days from the LH surge and it does not act differently when luteolysis occurs early, inasmuch as it always has to recover from a fall in progesterone to renew the state of estrus, I wonder whether Dr. Soules would comment about a classic homeostatically oriented hypothesis that would theorize that follicular recruitment and subsequent luteal function would if anything be more robust after a short, insufficient luteal phase.

In summary, Dr. Soules has achieved remarkable results in the delineation of luteal phase deficiency. His careful, meticulous studies led him to reason that decreased progesterone in the luteal phase might alter LH pulsatile secretion in the subsequent follicular phase. The idea constituted a brilliant attempt to consolidate a pathophysiologic vision about a difficult question in a single study. Unfortunately, this did not turn out to be the case. Therefore decreased progesterone production in one cycle does not lead to recurrent luteal phase defects in normal women, implying important evidence that supports the complex cause of this syndrome. Thus we will await with great expectations his follow-up studies that may shed further light on the matter.

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Dr. James Schreiber, Chicago, Illinois. The hypothesis was that low progesterone would set off the next cycle to make it short. I was curious whether another way to approach this would be to use an antiprogestin such as RU 486, mifepristone, and block progesterone directly rather than perturbing centrally with the GnRH antagonist.

Dr. Daniel H. Riddick, Burlington, Vermont. I wonder whether you have looked at the converse of your experimental design. Have you taken women with luteal phase deficiency and supplemented the progesterone to normal levels in a deficient cycle and then studied the subsequent cycle with no treatment in those women?

Dr. Soules (Closing). I thank Dr. Schiff for a very thorough review of the article and his thoughtful questions.

The first question was in regard to the time course of effect of altering progesterone on LH secretion. The LH pulse generator (the gonadotropin generator) accelerates when progesterone is not present. On the basis of our studies and the literature, when progesterone levels decrease in the midluteal to late luteal phase, the LH pulse generator speeds up. The increase in the LH secretory takes place over 2 to 4 days.

He brought up a good point: we did not necessarily prove the null hypothesis by studying only six women. We could have studied more women and found that our hypothesis was actually true or we could have stud-

ied more susceptible women (e.g., infertile women) and may have found that our hypothesis was true. That is a good criticism. However, I happen to doubt that the study of more or different subjects would have changed our findings because we did not even see a trend in our data toward luteal phase deficiency in the menstrual cycles after induced luteolysis.

Dr. Schiff asked whether addition of LH in the luteal phase could prevent luteal phase deficiency. Yes, I believe it can. In fact, human chorionic gonadotropin supplementation is an acceptable therapy for luteal phase deficiency. It is not used very often; but in my experience, human chorionic gonadotropin does work.

The fourth question was provocative in regard to the pituitary and whether it had a homeostatic relationship with the ovary. His example was the pituitary-thyroid axis wherein the pituitary does everything it can to keep the end organ functioning when it fails. I think the pituitary ovarian axis functions in the same manner. In the late luteal phase when there is the normal decrease in progesterone, the gonadotropin pulse generator accelerates with a corresponding increase in the mean levels of LH and FSH. The hypothalamic pituitary unit is trying to rescue the corpus luteum, but the corpus luteum remains resistant. Therefore the pituitary does act in its usual homeostatic manner with the ovary as well.

The next question was whether we measured FSH in these particular patients. The answer is no, but I would like to allude to our 1989 study in which we intensively studied 10 women with spontaneous luteal phase deficiency. In that study, we looked at FSH every way we could to determine whether there were any changes in FSH. We looked at immunoactive FSH, bioactive FSH, follicular size, and estradiol levels. In these infertile women with spontaneous luteal phase deficiency, we could not find any evidence that abnormalities in FSH or follicular development were a part of the pathogenesis of luteal phase deficiency. However, it is clear in the literature that altering FSH or follicular development can cause luteal phase defect.

The next question was with regard to biospies. No, we did not obtain endometrial biopsy specimens because we think the integrated progesterone level provides a much more accurate diagnosis of luteal phase deficiency.

Dr. Schiff's next question was whether we could have induced a different type of luteal phase deficiency (low progesterone levels but with normal luteal length). This is the most common pattern we see in spontaneous luteal phase deficiency. It would have been nice to induce this pattern, but our experience with the Nal-Glu GnRH antagonist is that you can not be that precise. In fact, we experienced the opposite problem of luteolysis being induced too quickly, wherein we dropped the bottom out of the corpus luteum immediately in some dose studies that preceded this study. So I think more prolonged luteolysis would have been a nice study design, but this was beyond the capabilities of our study drug.

The next question concerned our hypothesis. Dr. Schiff theorized that if you suppressed the corpus luteum, you could give the follicular phase more time to develop and may see a more robust ovarian response in the next cycle. That is an interesting alternative hypothesis. The natural response of the hypothalamic-pituitary unit to decreased levels of progesterone is to raise circulating levels of LH and FSH that promote follicular development in the ensuing cycle. In formulating our hypothesis we reasoned that if the hypothalamus were exposed to less progesterone over a shorter period of time, we could find a supraphysiologic acceleration in the LH secretion pattern. In our research experience wherein we induced a supraphysiologic LH secretion pattern we induced luteal phase deficiency. Furthermore, I am not aware of a natural (physiologic) gonadotropin secretion pattern that leads to healthier, more robust follicles. We expected to find either inadequate or normal follicular development in response to luteolysis and we found the latter. It would have been fascinating to have found a more robust subsequent cycle but that was a less plausible hypothesis based on our knowledge of ovarian physiology and our research experience.

Dr. Schreiber asked about a different study design, wherein the effect on the next cycle could be examined after a partially blocking progesterone feedback effect on the hypothalamic-pituitary unit with the progesterone antagonist RU 486. At one time, we considered the use of RU 486 for this purpose but decided against it because we could think of no way to monitor whether we induced a complete or partial block. To mimic luteal phase deficiency only a partial progesterone block at the hypothalamus-pituitary is necessary.

Finally, Dr. Riddick asked whether we have treated women with luteal phase deficiency with progesterone to determine whether the pulse pattern is normal in the subsequent follicular phase. This proposed study is a logical corollary to the study I just presented and we plan to start the very study Dr. Riddick has proposed within the next year.

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Assessment of current International Federation of Gynecology and Obstetrics staging of vulvar carcinoma relative to prognostic factors for survival (A Gynecologic Oncology Group Study)

Howard D. Homesley, MD,[a] Brian N. Bundy, PhD,[b] Alexander Sedlis, MD,[c] Edgardo Yordan, MD,[d] Jonathan S. Berek, MD,[e] Antoine Jahshan, MD,[f] and Rodrique Mortel, MD[g]

Winston-Salem, North Carolina, Buffalo and Brooklyn, New York, Chicago, Illinois, Los Angeles, California, and Philadelphia and Hershey, Pennsylvania

Analysis of 588 patients with vulvar carcinoma delineated four risk groups by the proportional hazards model. Groin node status (laterality and number positive) and lesion diameter were the only two important independent prognostic factors. The 5-year relative survival rates were 98%, 87%, 75%, and 29% for the risk group categories of minimal (negative groin nodes and lesion diameter ≤2 cm), low (one positive groin node and lesion diameter ≤2 cm or negative groin nodes and fewer than two lesions ≤8 cm diameter), intermediate (negative groin nodes and lesion diameter >8 cm diameter, one positive groin node and lesion diameter >2 cm, or two unilaterally positive groin nodes and lesion diameter ≤8 cm), and high (three or more positive groin nodes or two bilaterally positive groin nodes), respectively. Applying the International Federation of Gynecology and Obstetrics staging (1988) to these data discriminated risk of death (caused by recurrent vulvar cancer); the 5-year rates were 98%, 85%, 74%, and 31% for stages I, II, III, and IV, respectively. However, within International Federation of Gynecology and Obstetrics stage III there were 47 low-, 95 intermediate-, and 28 high-risk patients with relative survivals of 95%, 74%, and 34%, respectively. Overall, this assessment validates current International Federation of Gynecology and Obstetrics vulvar carcinoma staging, but further refinements are warranted in stage III. (AM J OBSTET GYNECOL 1991;164:997-1004.)

Key words: Prognostic factors, groin node surgical status, tumor size, carcinoma of the vulva, staging

Two prospective clinical trials in vulvar carcinoma, one to evaluate treatment and the other to assess the significance of surgicopathologic staging findings were activated by the Gynecologic Oncology Group in 1977. The prospectively randomized treatment trial of patients with positive groin nodes ended in 1984 and demonstrated that groin plus pelvic radiation therapy was superior to pelvic node resection after radical vulvectomy and groin dissection.[1] From the surgicopathologic study, predictive factors for groin node status in patients with superficial vulvar lesions (tumor thickness ≤5 mm) has been detailed.[2]

Evaluation of the clinicopathologic factors of patients with vulvar carcinoma in the above staging study for prognostic significance for survival are presented. In addition, by classifying the Gynecologic Oncology Group patients into four risk groups for survival, an assessment can be made of the validity of the new surgical staging for vulvar carcinoma formulated by International Federation of Gynecology and Obstetrics (FIGO) in 1988 (Table I).[3]

Material and methods

Only patients with previously untreated squamous cell carcinoma of the vulva, where radical vulvectomy sufficed to resect the primary lesion, were eligible. All patients signed an informed consent form.

From the Section on Gynecologic Oncology, Department of Obstetrics and Gynecology, Bowman Gray School of Medicine of Wake Forest University,[a] Roswell Park Cancer Institute,[b] the Department of Obstetrics and Gynecology, State University of New York, Downstate Medical Center,[c] the Section of Gynecologic Oncology, Rush-Presbyterian St. Luke's Medical Center,[d] the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of California Los Angeles School of Medicine, and Jonsson Comprehensive Cancer Center,[e] the Division of Oncology, Department of Obstetrics and Gynecology, Thomas Jefferson College,[f] and the Department of Obstetrics and Gynecology, The Milton S. Hershey Medical Center of Pennsylvania State University.[g]

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6/6/27333

Table I. FIGO staging for vulvar carcinoma (1988)[3]

Stage	*Findings*
I	Tumor ≤2 cm diameter with no nodal metastasis and confined to vulva and/or perineum
II	Tumor >2 cm diameter with no nodal metastasis and confined to vulva and/or perineum
III	Tumor of any diameter with: (1) adjacent spread to lower urethra and/or vagina or anus and/or (2) unilateral regional lymph node metastasis.
IVA	Tumor invasion of any of the following: upper urethra, bladder mucosa, rectal mucosa, pelvic bone, and/or bilateral regional node metastasis
IVB	Any distant metastasis including pelvic lymph nodes

Table II. Frequency and relative survival by groin node status

Node status	*No.*	*%*	*Relative survival at 5 yr (%)*
Negative	385	65.5	90.9
Positive	203	34.5	57.2
TOTAL	588	100.0	
No. positive			
1-2	125	62.5	75.2
3-4	40	20.0	36.1
5-6	19	9.5	24.0
≥7	16	8.0	0.0
TOTAL POSITIVE*	200	100.0	
Laterality			
Unilaterally positive	141	69.5	70.7
Bilaterally positive	62	30.5	25.4
TOTAL POSITIVE	203	100.0	

*Three patients had an undetermined number of positive nodes.

The surgical technique of Parsons and Ulfelder[4] was the standard for all investigators, but individual discretion decided the type of groin incision. Most patients with positive groin nodes were entered into the randomized trial of surgery only versus surgery plus groin and pelvic radiation.

Representative pathology slides were centrally reviewed by the Gynecologic Oncology Group Pathology Committee for uniform pathologic review of location, histologic grade, and number of positive nodes. Evaluation of histologic differentiation of vulvar lesions by Gynecologic Oncology Group criteria, as well as the basis for the most reproducible method for assessment of tumor invasion by measurement of tumor thickness, has previously been presented.[2]

Table III. Frequency and relative survival by node laterality for patients with a unilateral vulvar lesion

Groin node status	*No.*	*%*	*Relative survival at 5 yr (%)*
Negative	201	70.0	91.5
Ipsilaterally positive only	62	21.6	75.0
Contralaterally positive only	8	2.8	26.7
Bilaterally positive	16	5.6	30.5
TOTAL	287	100.0	

Cox's proportional hazards model[5] was used to determine the simultaneous effects of the prognostic factors on survival and relative survival with the likelihood ratio test. A standard step-up procedure identified all independently significant prognostic factors.[6] All medians and life tables were computed with the product-limit estimate by Kaplan and Meier.[7] Survival was defined as the time from study entry to the date of death or date last seen. Relative survival is the time from study entry to date of death or date last seen where deaths from causes other than recurrent vulvar carcinoma were withdrawn alive (censored) in the life-table analysis.

In this article significance refers to statistical significance computed by means of a two-sided statistical test where the p value is ≤ 0.05. Positive nodes, nodal involvement, and nodal spread refer to lymph nodes containing metastases.

The clinical and pathologic factors analyzed for survival were number of positive groin nodes, lesion diameter, surgical margins positive for invasive carcinoma, differentiation of tumor, clinical groin node status, age, lesion location, tumor thickness, and capillary–lymphatic space involvement.

Results

Of 637 patients entered into the study from 1977 to 1984, 49 were deemed ineligible, primarily because of incomplete surgery or inadequate pathologic study, leaving 588 evaluable patients available for analysis.

The surgical extent of metastases in the groin nodes ($p < 0.00001$) was by far the single most important prognostic factor for survival (Table II). Not only was the presence of positive groin nodes associated with a poor prognosis, but the number of positive nodes and whether there was unilateral or bilateral metastases further defined the risk of death. The contribution of the number of positive nodes was powerful. Those with two, four, and six positive nodes experienced 3.1, 6.2, and 11 times the risk of death from cancer compared

Table IV. Frequency and relative survival by tumor diameter and groin node status

	Surgical groin node status					
	Negative nodes			*Postive nodes*		
Tumor diameter (cm)	*No.*	*%*	*Relative survival at 5 yr (%)*	*No.*	*%*	*Relative survival at 5 yr (%)*
≤2	154	40.4	97.9	36	18.1	78.6
2.1-3	120	31.5	90.5	55	27.6	59.1
3.1-4	37	9.7	75.5	44	22.1	56.8
≥4.1	70	18.4	81.5	64	32.2	44.8
TOTAL*	381	100.0		199	100.0	

*Four patients with negative nodes and four patients with positive nodes had unknown lesion diameter.

with the patients with negative nodes, respectively. Bilateral involvement increased the risk by 80% (i.e., relative risk = 1.8). Clinically suspicious or positive nodes (if confirmed to be pathologically positive) increased the risk 50%.

Considering just those patients with unilateral vulvar lesions, contralateral groin metastasis dramatically decreased relative survival (Table III). There were eight (2.8%) patients with contralaterally positive groin nodes and ipsilaterally negative groin nodes.

Multiple regression analysis of relative survival was used to identify the significant independent risk factors. After the number of groin nodes with metastases (the most important risk factor, $p < 0.00001$) was entered into the model, the next most significant ($p = 0.0005$) factor was tumor diameter. Larger diameter tumors were indicative of a lower relative survival and were associated with a greater extent of groin node involvement (Table IV). Comparing small lesions (≤2 cm) with larger lesions (2.1 to 8 cm) yields an improved relative survival between 9 and 18 percentage points for the four categories of extent of groin node involvement (Table V).

The only other independent risk factor was clinical node status ($p = 0.05$). Since the increase in risk associated with clinically suspicious or positive groin nodes was only 60%, it was not useful in discriminating among the four risk groups defined below.

Various other surgicopathologic characteristics were evaluated for survival significance while adjustment for the extent of groin node metastasis and lesion diameter was done. Lesion location, Gynecologic Oncology Group histologic differentiation, standard tumor grading, tumor thickness, status of microscopic resection margins, capillary or lymphatic space involvement, and age were not significant for survival.

A portion of patients was entered on the Gynecologic Oncology Group treatment protocol. The regimens of radiation therapy versus pelvic node dissection, randomly assigned, were evaluated in the model. The beneficial impact of radiation therapy was statistically significant ($p = 0.02$, one-sided test), which is compatible with the published results.[1]

A distinction needs to be made between the factors that can be used to predict nodal status and factors that predict survival (Table VI). Clinical node status, Gynecologic Oncology Group grade, and capillary or lymphatic space involvement were all highly significant ($p < 0.0001$) as predictors of positive groin nodes. Tumor thickness ($p = 0.003$) also was highly prognostic. The odds ratio of increased risk for nodal involvement for age increased fivefold between ages 55 and 65, whereas a 75-year-old woman was 13 times more likely to have positive groin nodes than a 55-year-old woman.

Table VII details the four risk-group assignments with the predicted relative survival on the basis of the two major independent survival determinants of groin node status ($p < 0.00001$) and maximum diameter of the primary tumor ($p = 0.0005$).

The minimal risk group (27%) contains essentially these same patients as currently FIGO stage I. The largest group (40%) of patients are at low risk for recurrence. The low-risk group has lesions either 2.1 to 8 cm in size or ≤2 cm in diameter with only one positive node. The intermediate risk group (18%) is defined by large tumors (>8 cm), larger lesions with a positive node (>2 cm in diameter and one positive node), or two unilaterally positive nodes in a tumor ≤8 cm. The high-risk group (15%) is categorized by large lesions (>8 cm) with two unilaterally positive nodes, three or more positive nodes, or bilaterally positive nodes. Current FIGO staging does not consider a combined higher risk for number of nodes and tumor diameter.

Fig. 1 displays both the relative survival curves of the four Gynecologic Oncology Group risk groups on the basis of this statistical analysis and the 1988 FIGO staging system applied to the same study population. FIGO staging does discriminate relative survival; 5-year rates were 98%, 85%, 74%, and 31% for stages I, II, III, and IV, respectively.

Table V. Relative survival by tumor diameter and surgical groin node status

Tumor diameter (cm)	Negative nodes		No. of positive groin nodes							
			One		Two unilateral		Three or more or two bilateral		Total	
	RS (%)	No.	RS (%)	No.	RS (%)	No.	RS (%)	No.	RS (%)	No.
≤2	97.9	154	94.4	18	88.9	9	38.1	9	94.4	190
2.1-8	86.9	214	76.6	61	70.5	18	28.9	72	73.3	365
>8	65.8	13	66.7	3	50.0	3*	0.0	3	55.7	22
TOTAL	90.9	381	79.7	82	74.0	30	29.0	84	90.9	577†

RS, Relative survival at 5 years.

*Last patient in this cell was withdrawn at 14.5 months.

†Three patients had an undetermined number of positive nodes and eight had unknown lesion diameter.

Table VI. Independent risk factors* for groin node metastasis

Factor	p Value
Clinical node status	<0.0001
Gynecologic Oncology Group grade	< .00001
Capillary–lymphatic space involvement	<0.0001
Tumor thickness	0.003
Age	0.04

*These were based on the logistic model.

Of the 176 FIGO stage III patients, the Gynecologic Oncology Group risk designations would be six patients with minimal risk, 47 with low risk, 95 with intermediate risk, and 28 with high risk; relative survivals at 5 years were 100%, 95%, 74%, and 34%, respectively (Fig. 2). This dichotomy is due to differences in definition, specifically FIGO stage III patients with one positive node are considered low risk, whereas those with three or more unilaterally positive groin nodes are high risk with death rates more on par with stage IV patients.

Comment

In 1988 FIGO staging for vulvar carcinoma was revised to include surgical findings. Since a retrospective analysis of a large series of vulvar cancer patients was not available, the current study using the Gynecologic Oncology Group data base, aimed both to delineate the independent survival factors and to assess the validity of the new staging system.

Of all the surgicopathologic findings analyzed that might affect survival, only two factors were found to be independently significant—the extent of disease in the groin nodes and the tumor diameter.

The current study, using the Gynecologic Oncology Group vulvar carcinoma data base, allows extensive scrutiny of the validity of the newly adopted FIGO staging system. Since the new system is a surgically based system that relies heavily on surgical status of the groin lymph nodes, it was no surprise that there was a correlation between stage and survival. Unfortunately, in FIGO stage III the survival ranged between 34% and 100%, depending primarily on the number of positive groin nodes. As emphasized in these current findings, the number of positive nodes is a powerful predictor of outcome and should be included in a vulvar staging system.

Table VII. Gynecologic Oncology Group risk groups for vulvar carcinoma

Risk group*	Surgical findings†	Relative survival at 5 yr (%)	No.‡
Minimal	Tumor ≤2 cm diameter and negative nodes	97.9	154
Low	Tumor 2.1 to 8 cm diameter and negative nodes Tumor ≤2 cm diameter and one positive node	87.4	232
Intermediate	Tumor >8 cm diameter and negative groin nodes Tumor >2 cm diameter and one positive node Tumor ≤8 cm diameter and two unilaterally positive nodes	74.8	104
High	Tumor >8 cm diameter and two unilaterally positive nodes Three or more positive nodes Bilaterally positive nodes	29.0	87

*Evaluation was based on proportional hazards model.

†Findings for nodes were based on surgical pathologic status of groin nodes.

‡Three patients had undetermined number of positive nodes and eight had unknown lesion diameter.

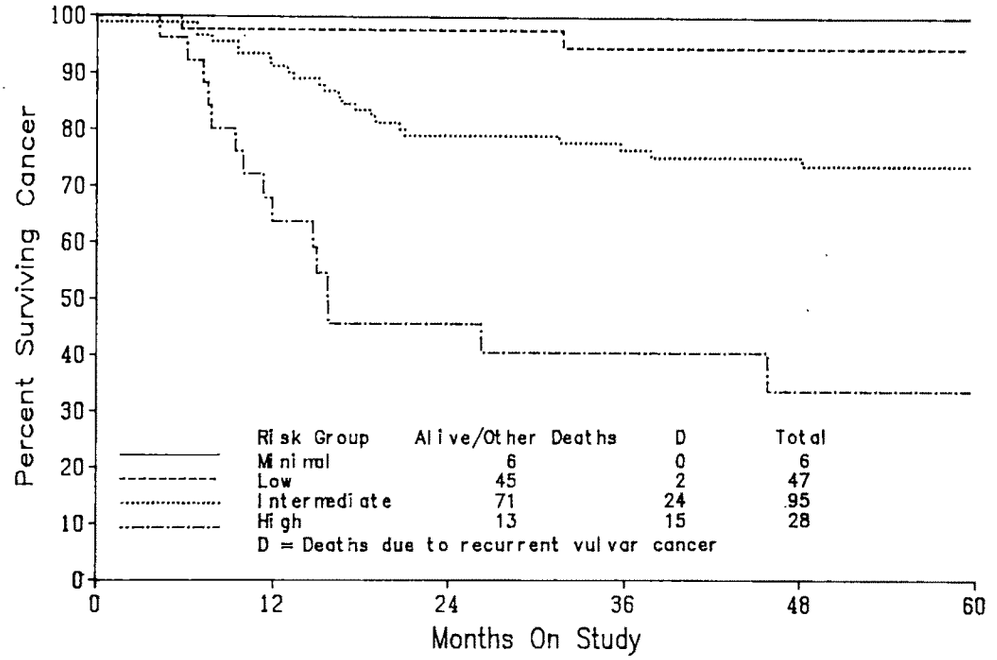


Fig. 1. Relative survival by FIGO stage (1988) versus Gynecologic Oncology Group risk groups, on the basis of the Cox model.

Likewise, the other independent risk factor of increasing diameter of tumor should be specified in a staging system. Lesions up to 8 cm in diameter are low risk as long as groin nodes are negative; however, even patients with negative groin nodes and lesions >8 cm in diameter incur intermediate risk of recurrence.

In combining the two independent risk factors, it is notable that with two unilaterally positive groin nodes (FIGO stage III) and a lesion >8 cm in size the patient is at considerably increased risk for recurrence compared with that of the patient with a lesion ≤8 cm and two positive groin nodes. This study included only three patients with this combination of risk factors. The major fallacy in the current FIGO staging is that there is no consideration in stage III for either increasing lesion diameter or number of positive nodes.

Adjacent spread to the lower urethra, vagina, or anus is categorized as stage III, or high risk, in the new FIGO staging system; yet this disease characteristic was not significant in the regression model ($p = 0.40$). In this study the local site of the lesion did not influence survival. However, this investigation was restricted to those patients with disease deemed resectable by radical vulvectomy and bilateral groin node dissection. This restriction could have eliminated the patients at highest risk of death, such as those with bladder or rectal extension or distant metastasis.

In patients with a unilateral primary lesion (49% of all patients) there was a high relative survival (92%) with negative groin nodes. Survival decreased markedly with ipsilateral groin node involvement (75%) and more drastically with bilateral groin spread (31%). This would justify placement of patients with bilateral regional node metastasis into high risk FIGO stage IVA. Patients with only a contralaterally positive groin node had a low relative survival (27%). However, the contralaterally and bilaterally positive groin node group comprised only 8% of patients with unilateral lesions.

The clinical node status, lesion location, tumor thickness (depth of invasion), Gynecologic Oncology Group differentiation of tumor, standard tumor grade, microscopic resection margins, capillary or lymphatic space involvement, and age in this study had no independent impact on relative survival.

The most powerful prognostic indicator was the number of positive groin lymph nodes. A high relative 5-year survival of 91% is anticipated in negative groin node patients, compared with 75% for patients with one or two positive groin nodes. All of the 16 patients with seven or more positive groin nodes were dead of disease at 5 years. Even the percent of positive groin nodes predicted a poor prognosis when >15% of removed nodes were positive.

The prior Gynecologic Oncology Group report on superficial vulvar lesions dealt primarily with the significance of tumor thickness as it related to depth of invasion and the other predictors of groin node status.[2] Capillary or lymphatic space involvement, Gynecologic

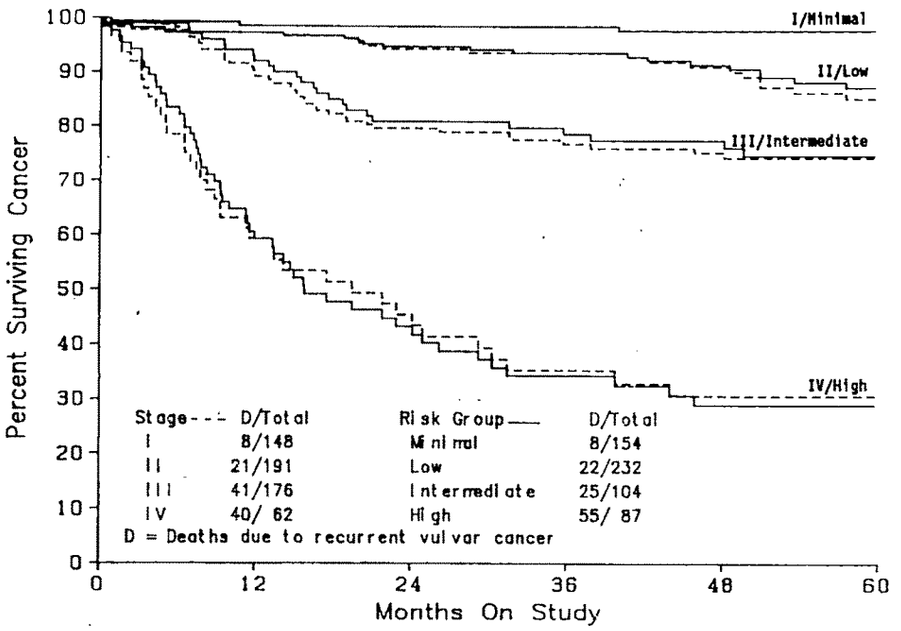


Fig. 2. Relative survival by Gynecologic Oncology Group risk groups, for FIGO stage III patients only.

Oncology Group tumor differentiation, lesion location, and clinical node status, along with tumor thickness, are all predictive of actual groin node status but not survival in patients with superficial lesions.

For this entire data base, in decreasing order, clinical node status, Gynecologic Oncology Group differentiation, capillary or lymphatic space involvement, tumor thickness, and age were the only predictors of groin node status. Nevertheless, only the number of positive groin nodes and tumor diameter are independent prognostic factors for survival that can be precisely used to specify relative risk and are highly predictive of survival.

Overall, the recently revised FIGO vulvar staging system allocates patients into proper risk categories because it is based on surgical staging. Unfortunately there are low-risk patients within stage III with one positive node that may be overtreated, as well as extremely high-risk patients with ≥3 positive nodes who have a poor prognosis.

The four Gynecologic Oncology Group risk groups constructed from the multiple regression analysis can serve as the basis for comparison of any proposed vulvar staging system because it is based on a comprehensive statistical appraisal of a vast number of patients with uniform staging. The two independent factors of groin node status and tumor diameter, when combined, are very powerful in discriminating risk of tumor death. Modifications that are based on Tables V and VII would improve the new vulvar staging system.

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Discussion

DR. JOHN ISAACS, Chicago, Illinois. Dr. Homesley and his Gynecologic Oncology Group colleagues have presented a thorough analysis of 588 patients with vulvar cancer. In their initial report they demonstrated in 114 patients that radical vulvectomy, groin dissection, and pelvic radiation gave a better 5-year survival than radical vulvectomy and bilateral groin and pelvic lymph node dissection.

In this presentation the same 114 patients, plus those who have been added since that publication, are being evaluated as to survival on the basis of the 1988 revision of the FIGO staging. The FIGO revision takes into consideration surgicopathologic findings, presumably to give the clinician a better idea of the chances for survival. In the 1988 FIGO staging, however, unilateral lymph node involvement places the patient into stage III regardless of the number of nodes involved. Likewise, if the patient has bilateral groin lymph node involvement, she is categorized into stage IV. Additionally, the FIGO revision does not take into consideration tumor size or diameter.

The authors' concern is that in those patients with stage III disease there is a wide variety of salvage rates, depending on lesion size and the number of positive lymph nodes, whether unilateral or bilateral. It is their opinion that some further refinements of stage III would allow for a more accurate prediction of survival, particularly since some of their stage III patients had a 100% 5-year survival.

It should be pointed out, however, that there is a small subset of stage III breast cancers that have a much better prognosis than most stage III breast malignancies. These are patients with histologically negative axillary nodes and a locally advanced breast cancer. In spite of this known fact, neither the American Joint Committee nor the International Union Against Cancer has suggested a revision of breast cancer staging.

The authors have done an excellent job of analyzing several factors that might influence survival. In so doing they have clearly shown that the number of lymph nodes involved is a powerful indicator of the chances for survival. As the number of positive nodes increases, survival decreases. This seems to apply whether the lymph node involvement is unilateral or bilateral. The other significant prognostic factor noted by the authors was tumor diameter. As the tumor diameter increases, the survival rate drops.

Dr. Homesley states that the statistical analysis eliminated other prognostic factors such as lesion location, degree of differentiation, etc. It is not entirely clear, at least to me, how these factors were eliminated since they did influence the possibility of lymph node involvement.

I would like to ask Dr. Homesley several questions to clarify this excellent presentation. First, do you believe that all 588 cases included in this report followed the precise surgical technique as outlined in the 1968 edition of Parsons and Ulfelder's *Surgical Atlas*? The operation described was quite extensive, and I believe many gynecologic oncologists have modified the dissection considerably since the 1968 edition. The authors stated that only the type of groin incision was left to the discretion of the participating surgeons. If the criteria were not followed precisely, however, alteration in the extent of the surgery could have had an effect on their statistics. One way of determining if the protocol was followed faithfully would be to ascertain if the number of nodes removed in each patient was fairly constant. Do you know the answer to this query?

Secondly, could you expand a bit on how you eliminated the lesion location and histologic differentiation as not being significant for survival? Statistics aside, I would have difficulty believing that a poorly differentiated tumor located in the clitoral area, for example, would not make a difference, nor can I understand that leaving cancer on the surgical margins would have no bearing on the ultimate survival.

Last, how many patients with diseased groin nodes were not entered into the randomized trial of surgery versus surgery plus radiation? In Material and methods it is stated that most patients with positive nodes were entered into the randomized trial of surgery versus surgery plus radiation. Under Results, however, it is stated that only a portion of patients was entered into the Gynecologic Oncology Group treatment protocol. Which was it?

In closing, is further redefinement of the current FIGO staging of vulvar carcinoma really necessary so

soon after its introduction and before further studies have been undertaken?

Dr. William T. Creasman, Charleston, South Carolina. I enjoyed the evaluation very much. I take it that the real concern is stage III, but did I understand correctly that you had eliminated all patients currently in stage III because of adjacent organ location? If that is the case, would that have affected your overall evaluation for stage III?

Dr. Homesley. As Dr. Isaacs may or may not know, all Gynecologic Oncology Group patients who have surgical management are reviewed by a separate committee called the Gynecologic Management Committee. This is a group of gynecologic oncologists who peruse the operative report and sometimes the discharge summaries, as well as look at pathology reports to determine whether the patient met the surgical criteria for entry. This is intense quality control. So I can confirm only that all these cases were carefully reviewed and they met the basic criteria for protocol entry.

As far as the types of groin incisions, morbidity is currently being evaluated. There could be differences in survival between these groups, but here again I think the dominating prognostic feature is the number of positive groin nodes.

No, we did not look for or have any minimum number of nodes for quality control in the groin dissection. We could look at that and determine how many nodes were removed in how many patients and how often the nodes were positive. A statistical evaluation could be done, but I doubt if it would be significant.

There is some confusion about lesion location, and I put this in the manuscript because there is a difference between what is predictive of groin node status and what is prognostic for survival. I think this should be stressed.

There are five predictive factors for groin node status—clinical node status, tumor grade, capillary or lymphatic space involvement, tumor thickness, and age. These are not prognostic factors for survival.

For example, if there is a clinically suspicious node in a patient with a Gynecologic Oncology Group grade 4 tumor that has capillary and lymphatic space involvement in the primary tumor, as well as tumor thickness of 10 or 15 mm, this patient is going to have a worse prognosis because the combination of all these factors is predictive of groin node metastasis from ages 55 to 75 and the relative risk increases 10 or 15 times.

If you're going to do conservative surgery, you have to take all of these predictive factors into account, on the basis of the combined probabilities of the likelihood of groin node metastasis and subsequent prognostic findings. The Gynecologic Oncology Group is conducting a clinical trial that is based on the current findings in which patients with lesions ≤2 cm with negative vascular and lymphatic space involvement and tumor thickness <5 mm are entered into a prospective trial of conservative surgery—hemivulvectomy and ipsilateral groin dissection. Thirteen percent have recurrence of disease and 9% have died. Thus there is a 91% survival with conservative surgery in a restricted group of patients who had favorable clinical and pathologic features.

As for surgical margins, we only dealt with microscopic margins because, as part of the eligibility requirements for the study, the lesions had to be cleared grossly. Even if margins were microscopically positive, it was not frequent enough to affect survival in this large series of patients.

Is it necessary to modify FIGO (1988) vulvar staging? I'm not saying it's necessary to change, but I think before a staging system is altered, it's necessary to take a sizable data base and use statistical methods for computation and prediction of the outcomes. With the large Gynecologic Oncology Group database, I think we can conclude that there will be heterogeneous survivals in stage III patients. If you want to have that, that's fine, but you will be forced to base your prognosis and treatment on the Gynecologic Oncology Group risk groups and not the FIGO staging.

FIGO (1988) vulvar staging is for standardized reporting. It also has some prognostic value, but for treatment I think you may have to use the Gynecologic Oncology Group predictors of groin node status to plan therapy and rely on the Gynecologic Oncology Group risk groups to predict outcome.

Dr. Creasman, I don't really think that there are all that many patients seen in the United States who have adjacent organ spread to justify this as a staging factor. Patients with adjacent site spread (vagina, urethra, and anus) weren't eligible for this study because, at the time we initiated it, it was a clinical pathologic staging of patients who could undergo resection by radical vulvectomy and bilateral groin dissection.

As it turns out, and hopefully not to confuse you, there were six FIGO stage III patients in our population who had lesions ≤2 cm but who had microscopic involvement of the vagina, lower urethra, or anus; all six patients survived. They actually were at minimal risk because they didn't have large lesions or groin node involvement. The two dominant prognostic features for survival are lesion size and number of positive groin nodes, and these two factors should be the basis for a vulvar staging system.

Comparison of pulsatile and continuous ritodrine administration: Effects on uterine contractility and β-adrenergic receptor cascade

Steve N. Caritis, MD, Jye Ping Chiao, MS, and Pam Kridgen, BS
Pittsburgh, Pennsylvania

In this study we compare the uterine contractility and β-adrenergic receptor effects of identical doses of ritodrine administered intermittently or continuously for 24 hours in pregnant sheep. Ritodrine was administered intravenously to five animals as a pulse, 16 μg/kg every 1.5 hours, whereas five other animals received ritodrine as a continuous infusion of 0.18 μg/kg/min. Ritodrine plasma concentrations at steady state were comparable in both groups and averaged 18 ng/ml. Animals receiving ritodrine pulses demonstrated no alteration of myometrial β-adrenergic receptors or adenylyl cyclase activity, and ritodrine inhibited oxytocin-induced contractility comparably at 4 and 24 hours. Animals receiving ritodrine continuously had a significant decrease in myometrial β-adrenergic receptors and adenylyl cyclase activity, yet ritodrine inhibition of oxytocin-induced uterine contractility was sustained for 24 hours. Oxytocin receptors were not affected by ritodrine administration and were similar in both groups. At the dose studied, oxytocin-induced contractions are comparably inhibited by ritodrine for 24 hours whether the drug is given continuously or in a pulsatile fashion. (AM J OBSTET GYNECOL 1991;164:1005-12.)

Key words: Ritodrine, pulsatile, desensitization, down regulation

Berg et al.[1] have demonstrated a reduction in myometrial β_2-adrenergic receptors in pregnant women treated with β_2-adrenergic receptor agonist. These investigators, however, did not evaluate changes in contractility that might occur as a consequence of the reduction in β-adrenergic receptor density. In this regard we have demonstrated that an infusion of ritodrine (1 to 4 μg/kg/min) to pregnant sheep for 24 hours results in a loss of myometrial β_2-adrenergic receptors and a decrease in isoproterenol-stimulated adenylyl cyclase activity in myometrial membranes.[2] These receptor changes are associated with a reduction in the effectiveness of ritodrine inhibition of oxytocin-induced uterine contractility. These findings suggest that the effectiveness of β-adrenergic agonists is self-limited, and indeed clinical studies suggest that the labor-inhibiting effect of β-agonists is short-lived.[3] In many species endogenous substances are released in a pulsatile fashion and the biologic response to the substance is not obtunded with continued exposure of the receptor to its agonist. Ke et al.[4] demonstrated that spontaneous myometrial contractility in vitro could be inhibited for prolonged periods by intermittent exposure to isoproterenol. Continuous exposure to the agonist, on the other hand, led to only transient inhibition of myometrial contractility. We undertook the present study to evaluate the in vivo effect of pulsatile β-agonist administration on myometrial β-adrenergic receptors and inhibition of oxytocin-induced myometrial contractility.

From the Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, Magee-Womens Hospital.
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Reprint requests: Steve N. Caritis, MD, Magee-Womens Hospital, Forbes and Halket St., Pittsburgh, PA 15213.
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Material and methods

Eighteen sheep at gestational ages of 110 to 125 days (time-dated) were anesthetized with general anesthesia (nitrous oxide/oxygen 1:1 and 0.5% halothane). After laparotomy we obtained two full-thickness uterine biopsy specimens of approximately 1 × 6 × 0.2 cm, weighing 3 to 6 gm. Samples were taken from the ventral surface of the pregnant uterine horn away from the cervix. A catheter was placed in the amniotic cavity and the uterine incision was closed. Polyvinyl catheters were also placed in a maternal pedal artery and vein and in 13 animals in an auricular vein. The arterial catheter was advanced to the abdominal aorta just above the common uterine artery. All catheters were tunneled subcutaneously and stored in a plastic pouch that was sewn to the animal's flank.

Study design. Eight animals were used to determine a pulse frequency and dose of ritodrine that would inhibit oxytocin-induced contractility without significantly reducing myometrial β-adrenergic receptors. We used ritodrine doses of 4 to 16 μg/kg injected every 30 to 120 minutes into the auricular venous catheter. The lowest doses did not provide adequate inhibition,

whereas the highest doses inhibited oxytocin-induced contractions but also caused a loss of myometrial β-adrenergic receptors (data not shown). A dose of 16 μg/kg given every 90 minutes for 24 hours provided adequate inhibition of oxytocin-induced contractility without causing a loss of myometrial β-adrenergic receptors. We therefore used this dosage (256 μg/kg/24 hours) for five animals. For the control group of animals (n = 5) we used the same total dosage (256 μg/kg/24 hours) but administered the drug as a constant infusion. For the 10 study animals we waited 2 to 4 days after surgery before initiating our study. The animals were brought to a study room in a transport cage and were allowed food and water as desired. After a baseline recording of maternal heart rate and uterine activity, all animals received a bolus of 500 mU of oxytocin, which was injected into the femoral artery. Thirty minutes after the oxytocin injection, five animals received ritodrine at a rate of 0.356 μg/kg/min for 30 minutes as a loading dose to rapidly achieve steady-state trough concentrations. This was followed by a maintenance infusion of 0.178 μg/kg/min for 24 hours (total maintenance dose, 256 μg/kg/24 hours). Five animals received three injections of ritodrine, 16 μg/kg every 2 minutes as a pulse through the auricular catheter to rapidly achieve steady-state trough concentrations. This was followed by injections of 16 μg/kg every 90 minutes for 24 hours. The total maintenance dose received in the second group of animals was also 256 μg/kg/24 hours. In the animals receiving ritodrine continuously, we injected 500 mU of oxytocin in the femoral arterial catheter after 4 and 24 hours of ritodrine infusion. In the animals receiving the drug in a pulsatile fashion, 500 mU of oxytocin was injected 60 minutes after the ritodrine pulses, which occurred at 3 and 22.5 hours after ritodrine was started. After 24 hours of ritodrine administration, all animals were killed with a lethal dose of Buthanasia-D (Schering). Myometrial tissue was removed from sites adjacent to the previous biopsy sites and the tissue was prepared as described below.

Tissue preparation. The myometrial tissue used in receptor density or adenylyl cyclase activity assays was frozen immediately in a methanol bath and stored at −70° C. The method for preparing tissue has been previously described.[2] In brief, the myometrial tissue was minced with scissors into 0.2 to 0.5 cm pieces and washed with sucrose buffer (0.25 mol/L sucrose, 1 mmol/L ethylenediaminetetraacetate, and 10 mmol/L Tris hydrochloride at pH 7.4). The tissue was then homogenized with 5 to 10 volumes of sucrose buffer at 4° C with a Brinkman Polytron device at setting No. 7 for 15 seconds, then centrifuged at 3000 g for 10 minutes. The supernatant was centrifuged at 20,000 g for 20 minutes. The pellet was suspended in sucrose buffer and centrifuged again as above. The pellet was resuspended in 0.05 mol/L Tris hydrochloride buffer, pH 7.4, with 0.9% sodium chloride to a final protein concentration of 2 to 3 mg/ml. Aliquots of myometrial membranes were then quickly frozen in a methanol bath and stored at −70° C until assayed within 60 days.

Measurement of β-adrenergic receptors, oxytocin receptors, and adenylyl cyclase activity

β-Adrenergic receptor assay. Myometrial membranes were thawed at room temperature and assayed for protein content by the method of Lowry et al.[5] Membranes (100 to 200 μg) in 0.05 mol/L Tris hydrochloride buffer were incubated in duplicate with 0.5 to 3.0 nmol/L [^{3}H]dihydroalprenolol (New England Nuclear) in a volume of 250 μl for 20 minutes at 30° C. Binding was at equilibrium under these conditions.[2] Nonspecific binding was determined with 10 μmol/L l-propranolol. Incubations were terminated by adding 3 ml of ice-cold Tris buffer that was quickly filtered through Whatman GF-C filters. The filters were washed with 5 ml of ice-cold Tris buffer three times, dried in an oven, placed in scintillation vials containing 5 ml of scintillation cocktail, and counted to 5 minutes by a Packard Tri-Carb scintillation counter (efficiency = 70%).

Oxytocin receptor assay. We used the method of Riemer et al.[6] to measure myometrial membrane oxytocin receptor density. We incubated 100 μg of membrane protein with 0.4 to 8.0 nmol/L [^{3}H]oxytocin (Sigma). Nonspecific binding (<20%) was determined from binding of [^{3}H]oxytocin to membranes in the presence of 1 μmol/L oxytocin.

Adenylyl cyclase activity. We used the competitive protein-binding method of Gilman[7] to measure the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP).[10] Membrane protein (20 to 40 μg) was incubated in duplicate for 15 minutes at 37° C with 50 mmol/L HEPES buffer, pH 7.4, 8 mmol/L potassium chloride, 1 mmol/L isobutyl methylxanthine, 3 mmol/L dithiothreitol, 0.4 mg/ml bovine serum albumin, 6 mmol/L magnesium chloride, 1 mmol/L of ATP, 0.25 mmol/L creatine phosphate, and 0.075 mg/ml creatine phosphokinase to a final volume of 300 μl. Reactions were terminated by adding 75 μl of 150 mmol/L acetic acid at room temperature. The membranes were centrifuged at 3000 g for 15 minutes. An aliquot of the supernatant containing cAMP was incubated with 5 to 10 μg cAMP-binding protein kinase and 0.05 μCi of [^{3}H]cAMP (New England Nuclear). Binding reached equilibrium by 2 hours; therefore all incubations were performed at 4° C for 2 hours. One milliliter of charcoal solution was added to each tube, which was centrifuged at 3000 g for 10 minutes. The supernatant was counted in a Packard Tri-Carb liquid scintillation counter after addition

of scintillation cocktail. We quantified cAMP by comparing the unknown samples with cAMP standards (1 to 24 pmol) prepared with the same reagents. Each microgram of cAMP-dependent protein kinase from bovine heart binds 0.12 pmol of cAMP at 40 nmol/L, pH 4 at 0° C. Others have found that cross-reactivities with other cyclic nucleotides are <1% that of cAMP.[7] The relationship of membrane protein concentration to cAMP production was linear (n = 0.996) from 2.8 to 57 μg protein. Interassay and intraassay variability was 13%. Basal adenylyl cyclase activity was not increased by isoproterenol without the presence of guanyl nucleotides. We therefore added 10 μmol/L 5'-guanylylimidodiphosphate to all incubations with isoproterenol. We also added 10 mmol/L sodium fluoride to some incubations without isoproterenol or guanyl nucleotides to evaluate postreceptor adenylyl cyclase activity.

Quantification of uterine activity. Uterine activity was measured by a Statham pressure transducer and recorded on a multichannel recorder. Uterine activity was quantified by integrating the area under the time–uterine pressure curve with the Bioquant II program (R & M Biometrics). We compared the area under the curve for a 10-minute epoch before oxytocin injection and again for 10 minutes after oxytocin injection. For the animals receiving ritodrine as a pulse, baseline uterine activity was recorded between 50 and 60 minutes after the ritodrine pulse. Oxytocin was injected at 60 minutes, and the uterine response was recorded from 60 to 70 minutes after the ritodrine pulse.

We have previously shown that in sham-operated animals receiving oxytocin pulses intermittently during a 24-hour infusion of saline solution there were no changes in β-adrenergic receptor density or adenylyl cyclase activity. Repeated pulses of oxytocin produced a reproducible myometrial response.[2]

Ritodrine measurement. Blood (10 ml) was collected in heparinized syringes before ritodrine infusion and during the ritodrine infusion preceding each injection of oxytocin. In animals receiving ritodrine in a pulsatile fashion, blood was also obtained at 45, 60, 65, and 70 minutes after ritodrine injection. Thus in the latter group four blood samples were obtained during the 25-minute epoch in which baseline activity was recorded and the uterine response to oxytocin was quantified. Samples were centrifuged at 1500 g for 10 minutes, and the supernatant plasma was frozen at −20° C until analyzed by high-performance liquid chromatography with μBondapak C_{18} reverse-phase column and electrochemical detection.

Statistical analysis. Changes in β-adrenergic receptor density, adenylyl cyclase activity, and area under the uterine pressure–time curve in the two groups were compared by two-way analysis of variance and the Newman-Keuls test. Time and treatment regimen were used as the two grouping factors for the two-way analysis of variance. All values are expressed as mean ± SD except in Fig. 1, where data are mean ± SE.

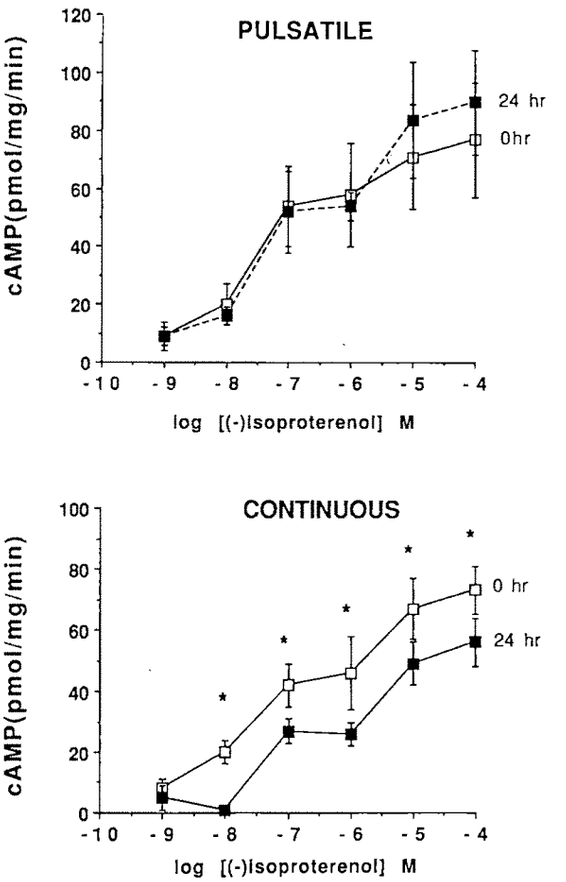


Fig. 1. Adenylyl cyclase activity in ovine myometrial membranes from animals receiving ritodrine by pulsatile *(top)* or continuous *(bottom)* infusion. Membranes obtained before ritodrine administration and after 24-hour ritodrine infusion were incubated with various concentrations of *l*-isoproterenol. Amount of cAMP produced is directly related to amount of adenylyl cyclase activity. *Asterisks* indicate significantly different values between 0 and 24 hours.

Results

The changes in myometrial β_2-adrenergic receptor quantity, agonist affinity (dissociation constant), and area under the uterine pressure–time curve in the two groups of animals are summarized in Table I. In animals receiving ritodrine in a pulsatile fashion no significant change in myometrial β-adrenergic receptor density or agonist affinity was demonstrated. Uterine response to oxytocin was reduced by 40% ($p < 0.05$). This degree of inhibition was maintained for the duration of ritodrine administration.

Animals receiving ritodrine continuously had a 42%

Table I. Uterine effects of ritodrine during pulsatile and continuous administration

	Pulsatile ($n = 5$)	*Continuous* ($n = 5$)
β-Adrenergic receptor density (fmol/mg)		
0 hr	286 ± 97	240 ± 80
24 hr	276 ± 107	140 ± 38*
Dissociation constant (nmol/L)		
0 hr	0.64 ± 0.01	0.58 ± 0.19
24 hr	0.62 ± 0.06	0.47 ± 0.17
Area under uterine pressure–time curve (cm^2)		
0 hr	15 ± 3	22 ± 7
4 hr	9 ± 2*	11 ± 5*
24 hr	9 ± 2*	12 ± 7*

*$p < 0.05$.

decrease in myometrial β-adrenergic receptors ($p < 0.05$) but no change in the dissociation constant. Oxytocin-induced contractility at 4 hours was inhibited by 50% ($p < 0.05$). In spite of the changes in β-adrenergic receptor density, the ability of ritodrine to inhibit oxytocin-induced contractility was not reduced after 24 hours of the infusion.

Fig. 1 shows the activation of adenylyl cyclase by various concentrations of *l*-isoproterenol. At concentrations >10 nmol/L, the adenylyl cyclase activity of myometrial membranes from animals receiving ritodrine continuously was significantly less than that of tissue from animals receiving the drug in a pulsatile fashion ($p < 0.05$). At a concentration of 0.1 mmol/L, adenylyl cyclase activity was 26% lower in animals receiving ritodrine continuously than in animals receiving ritodrine in a pulsatile fashion. Table II shows that basal adenylyl cyclase activity, sodium fluoride–stimulated adenylyl cyclase activity, and mean effective concentration of isoproterenol did not change significantly in either group as a consequence of ritodrine administration. Similarly, as indicated in Table III, oxytocin receptor density and dissociation constant were not altered by either regimen of ritodrine administration.

In animals receiving ritodrine at a constant rate, the concentrations at 4 and 24 hours were 15 ± 4 and 18 ± 5 μg/ml, respectively. Thus steady state had essentially been achieved by the fourth hour of infusion. In the animals receiving ritodrine pulses, the ritodrine concentration was measured four times between 45 and 70 minutes after a ritodrine pulse and averaged 14 ± 2 and 18 ± 3 μg/ml at 4 and 23.5 hours, respectively. These concentrations were similar to those seen with the continuous infusion protocol. There was little variation in the ritodrine concentration between the 45- and 70-minute time points. In the five animals the mean variation in ritodrine concentration at these two time points was 0.9 ± 2.0 ng/ml. Thus the ritodrine concentrations were relatively constant during oxytocin-induced uterine contractility.

Comment

This study has demonstrated that the pulsatile administration of ritodrine at the dose and frequency described does not significantly reduce myometrial β-adrenergic receptor density or adenylyl cyclase activity. Ritodrine inhibition of oxytocin-induced contractility is maintained for 24 hours. Continuous infusion of the same dose of ritodrine for 24 hours, however, results in a 42% loss of β-adrenergic receptors and a 26% reduction in isoproterenol-stimulated (0.1 mmol/L) adenylyl cyclase activity. Despite these receptor-mediated changes during continuous ritodrine infusion, there was no attenuation of ritodrine's ability to inhibit oxytocin induced contractility after 24 hours' infusion.

Our previous work has shown that the continuous infusion of high doses of ritodrine for 24 hours results in a reduction of myometrial β-adrenergic receptors, a loss of isoproterenol-stimulated adenylyl cyclase activity, and an attenuation of ritodrine inhibition of oxytocin-induced contractions.[2] These receptor effects were observed with plasma concentrations ranging from 45 to 164 ng/ml. In this study we demonstrated similar effects on β-adrenergic receptor density and isoproterenol-stimulated adenylyl cyclase activity with ritodrine concentrations averaging 18 ng/ml (range, 10 to 25 ng/ml). The lowest concentration of ritodrine required to reduce β-adrenergic receptor density and adenylyl cyclase activity has not been established. β-Adrenergic receptor loss in this study occurred at concentrations that minimally stimulate adenylyl cyclase activity, i.e., 30 ng/ml (unpublished data).

In spite of qualitatively similar effects of continuous ritodrine infusion on the β-adrenergic receptor and isoproterenol-stimulated adenylyl cyclase activity, the inhibitory effect of ritodrine on oxytocin-induced uterine contractions differed between this study and our previous study. In contrast to the previous study, the receptor effects described here were not accompanied by an attenuation of ritodrine inhibition of oxytocin contractility in this study. The reason for this differ-

Table II. Uterine effects of ritodrine during pulsatile and continuous administration

	Pulsatile (n = 5)	*Continuous (n = 5)*
Basal adenylyl cyclase activity (cAMP, pmol/mg/min)		
0 hr	17 ± 8	16 ± 5
24 hr	17 ± 7	17 ± 8
Sodium fluoride–stimulated (10 mmol/L) adenylyl cyclase activity (cAMP, pmol/mg/min)		
0 hr	192 ± 96	148 ± 38
24 hr	236 ± 77	153 ± 61
Agonist mean effective concentration for adenylyl cyclase activity (nmol/L)		
0 hr	60 ± 28	240 ± 269
24 hr	160 ± 110	440 ± 279

Cyclase activity in presence of 10 μmol/L *l*-isoproterenol.

Table III. Uterine effects of ritodrine during pulsatile and continuous administration

	Pulsatile (n = 5)	*Continuous (n = 5)*
Oxytocin receptor density (fmol/mg protein)		
0 hr	899 ± 383	1048 ± 381
24 hr	990 ± 270	1173 ± 684
Oxytocin receptor dissociation constant (nmol/L)		
0 hr	1.24 ± 0.55	1.15 ± 0.24
24 hr	1.49 ± 0.74	1.62 ± 0.67

ence is unclear. One possible explanation may be the quantitative differences in receptor effects between the two studies. Both the magnitude of the receptor loss (50% vs 42%) and the adenylyl cyclase response to 0.1 mmol/L isoproterenol (70% vs 26%) were greater in the previous study. Plasma ritodrine concentrations averaged 85 ng/ml in the previous study and only 18 ng/ml in this study. Quantitative comparisons between the two studies, however, are problematic. The number of animals in the two studies was small and substantial variation exists in receptor-associated effects between animals. Another possible explanation for the different uterine response is that the higher ritodrine concentrations in the previous study altered the hormonal milieu in such a fashion to make the myometrium more responsive to oxytocin. We measured oxytocin receptor density and agonist affinity in the present study, these were unaffected by ritodrine infusion. We did not, however, measure oxytocin receptors in our previous study. Additionally, ritodrine increases uterine production of prostaglandins.[8] This effect may be dose related and may account for the greater uterine responsiveness to oxytocin in our previous study. It is also possible that during continuous infusion of ritodrine, numerous intracellular processes that may be responsible for the process of desensitization may be affected to a greater degree with higher doses of ritodrine. For example, phosphodiesterase activity may be increased to a greater degree with higher ritodrine concentrations, resulting in more rapid degradation of cAMP.[9]

The decrease in β-adrenergic receptors after continuous ritodrine infusion was associated with a similar but somewhat smaller decrease in membrane adenylyl cyclase activity. This finding suggests that there are few "spare receptors" in ovine myometrium. The concept of spare receptors was developed to explain how adenylyl cyclase activity is maintained in many tissues despite a substantial decrease in β-adrenergic receptors.[10] This clearly was not the case in this study. The observation that adenylyl cyclase activity decreased by 26% while receptor decreased 42% suggests that the pool of spare receptors is small.

The dose-response studies with isoproterenol indicate an attenuation of response at all concentrations >10 nmol/L isoproterenol in animals receiving ritodrine continuously. Basal adenylyl cyclase activity, as well as fluoride-stimulated activity, was not affected, indicating that the reduced adenylyl cyclase response is not mediated through a decreased amount of adenylyl cyclase enzyme. Fluoride ion stimulates adenylyl cyclase, directly bypassing the receptor mechanism. A robust adenylyl cyclase response to sodium fluoride indicates that adenylyl cyclase itself is not affected by continuous ritodrine infusion.

Ke et al.[4] have shown that spontaneous myometrial contractility in vitro can be inhibited for prolonged periods with intermittent, but not continuous, exposure to β-agonists. Casper and Lye[11] administered ritodrine and isoproterenol to nonpregnant oophorectomized sheep and measured estradiol-induced uterine contractility. A continuous infusion of a β-agonist initially inhibited uterine contractility but the effect was short-

lived, lasting 6 to 10 hours. Intermittently administered isoproterenol (30 minutes on and 30 minutes off) inhibited uterine contractility for 13 hours. The greatest degree of inhibition occurred during the isoproterenol infusion; uterine contractility increased substantially during the 30-minute period that the infusion was stopped. In the study of Casper and Lye,[11] several regimens of intermittent ritodrine administration were not successful in maintaining uterine quiescence for long periods. Our success with ritodrine is likely attributable to the integration of pharmacokinetic and pharmacodynamic data specific to ritodrine. We selected a dose and pulse frequency that would keep trough plasma concentrations of ritodrine at <30 ng/ml. We have found that ritodrine stimulation of adenylyl cyclase activity is minimal at concentrations <100 nmol/L, i.e., 30 ng/ml (unpublished data). We supposed that receptor-mediated effects might be minimized if there were insufficient binding of ritodrine to β-adrenergic receptors to stimulate cyclase activity.

We were surprised to find that plasma ritodrine concentrations were similar in the two groups of animals. Theoretically, with the dosing regimens used in the study, trough ritodrine concentrations would be expected to be lower than the steady-state concentrations during a constant infusion of the same dosage. Ritodrine plasma concentration is dependent on drug clearance. Ritodrine clearance, however, can vary as much as threefold.[12] Since our study was cross-sectional, the similarity in ritodrine concentrations in the two groups is probably due to interanimal variation in ritodrine clearance. Had each animal received ritodrine by both regimens, we would have expected to demonstrate a somewhat lower trough plasma concentration in animals receiving the drug as a pulse. Such a study design, however, would have prevented an analysis of the myometrial receptor effects.

In many tissues of various species, continuous exposure of β-adrenergic receptors to β-agonists leads to a reduction in receptor number.[10] The agonist-receptor complex is phosphorylated and may be internalized, making it inaccessible to further agonist binding. It is unclear why receptors were not lost in those animals receiving the drug in a pulsatile fashion. Plasma concentrations at steady state were not significantly different in the two groups, suggesting that the different receptor effects in the two groups were not attributable to differences in plasma ritodrine concentrations. Plasma progesterone concentrations were not significantly altered by either drug administration regimen (data not shown). Therefore the different effect on β-adrenergic receptor density cannot be explained by differential effects of ritodrine on progesterone release or production. One possible explanation for these differential receptor effects is that the transiently high ritodrine concentration after a pulsatile injection inhibits the phosphorylation of the receptor, preventing its sequestration. Alternately, transiently high ritodrine concentrations may enhance transcription of new receptors.

The clinical implications of this study are numerous. It is clear that a loss of β-adrenergic receptor density does not mean that the clinical effectiveness of a β-agonist is lost. In view of the low ritodrine concentration required to reduce β-adrenergic receptors, it is likely that even with the lowest dose of ritodrine used to treat preterm labor, a marked loss of β-adrenergic receptors will occur.[13] Recently β-adrenergic receptor density in peripheral lympocytes in humans has been closely correlated with myometrial β-adrenergic receptor density.[14] Our findings suggest, however, that a reduction in receptors in either tissue does not necessarily mean a loss of tocolytic effectiveness.

Recently the β-agonist terbutaline has been administered in a pulsatile fashion to pregnant women to prevent recurrent preterm labor.[15] The dosing frequency and quantity were titrated to spontaneous uterine contractility and were not based on the pharmacokinetic properties of the β-agonist. There is no evidence that the recommended regimen prevents receptor loss or maintains tocolytic efficacy. Our preliminary studies in this report indicate that too frequent or too high a dose will cause a loss of receptors and a loss of tocolytic efficacy. Clearly, additional studies are required to define the dose response for β-adrenergic receptor loss, the decrease in adenylyl cyclase activity, and the loss of tocolytic effectiveness after pulsatile β-agonist administration.

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Discussion

DR. ROBERT C. CEFALO, Chapel Hill, North Carolina. In the United States, preterm labor and delivery still account for the majority of the neonatal morbidity and mortality, in spite of the implementation of preterm labor prevention protocols and the use of different classes of tocolytic agents. It appears that both the failure rate and side effects of certain tocolytic agents are related to the β-receptor/β-agonist interaction. During therapy with β-agonist drugs, recurrent preterm labor is believed to be secondary to a down regulation or desensitization of the myometrial β_2-adrenergic receptors, which leads to a state refractory to further tocolysis. Clinically, the limited duration of relaxant effect of β_2-adrenergic receptor agonist as a treatment of preterm labor makes this a relevant and important question. Caritis et al. attempted to clarify the mechanism behind the desensitization of the myometrial β_2-adrenergic receptor after exposure to a β_2-adrenergic receptor agonist. Desensitization of the β-adrenergic receptor system may come about by a decrease in the number of receptors or a decrease in the total adenylate cyclase activity. These desensitization mechanisms have been demonstrated in vitro and clinically after continuous exposure of adrenergic receptor stimulation. This phenomenon has led investigators and clinicians to consider the use of a pulsatile bolus of a β-agonist in an attempt to overcome the desensitization associated with continuous infusion.

The authors investigated and compared the in vivo effect of continuous β-agonist and pulsatile β-agonist administration on myometrial β-adrenergic receptor activity and inhibition of oxytocin-induced myometrial contractility in a near-term pregnant sheep model. Their findings indicate that the animals receiving ritodrine pulses demonstrated no alteration of myometrial β-adrenergic receptor activity, and ritodrine inhibited oxytocin-induced contractility comparably at 4 and 24 hours. The animals receiving ritodrine continuously had a statistically significant decrease in myometrial β-adrenergic receptor activity, yet the ritodrine inhibition of oxytocin-induced uterine contractility was sustained for 24 hours. Neither pulsed nor continuous administration of ritodrine affected oxytocin receptors. Thus, after rigorous scientific investigation that involved the pharmacokinetics of the β-agonist, sophisticated quantification of β-adrenergic receptor, oxytocin receptor, and adenylyl cyclase activity, we are left with more knowledge but further questions.

Stimulation and increase in density of β-adrenergic receptors in both pregnant and nonpregnant human or animal myometrium by β-adrenergic receptor agonists results in smooth muscle relaxation. This up regulation of β-adrenergic receptor and adenylate cyclase function may be regulated in part by local or systemic estrogen-progesterone levels.[1] In many species it is known that both local and systemic placental progesterone production are important for pregnancy maintenance and uterine relaxation. In pregnancy, local progesterone secretion results in functional adrenergic denervation of the myometrium, which may contribute to the observed up regulation of the β-adrenergic receptor function and increase in the number of β-adrenergic receptors and in adenylate cyclase activity contributing to myometrial relaxation and pregnancy maintenance.

In the pregnant ewe an extremely high plasma progesterone concentration is maintained until delivery. The concentration of estrogens in maternal plasma is low during most of the pregnancy and increases tenfold on the day before labor. Just before the onset of labor there is an alteration in the estrogen-to-progesterone ratio that may be involved in the sensitivity of the myometrium to the effects of oxytocin and prostaglandins.

I have three questions for Dr. Caritis. Is progesterone production affected by either pulsatile or continuous ritodrine administration? In view of the known progesterone and estrogen effects on β-adrenergic receptor activity, did the authors take into account that the quantification of myometrial adrenergic receptor and adenylate cyclase activity may have been affected by relative levels of estrogen and progesterone in the maternal circulation as well as locally, whether the myometrial biopsy specimen was taken over the cotyledonary site or between cotyledons? By the methods used, could the authors differentiate between the uterine smooth muscle membrane fraction and the smooth muscle membrane fraction from small-caliber blood vessels obtained in their myometrial biopsy specimens?

The authors are to be congratulated for attempting to further clarify β-adrenergic receptor mechanisms and classify the range of responses to β-agonist administration, looking for the lowest possible dose compatible with a clinical effect. It is only through a basic

understanding of these mechanisms that we can come closer to suggesting a therapeutic approach that can assist in the management of preterm labor.

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DR. THOMAS H. KIRSCHBAUM, Los Angeles, California. Dr. Caritis, I wonder if we aren't looking at the wrong source of intracellular energetics in this case. As I remember, there is some evidence in the human and in some animals that the conversion of ATP to cAMP is less important quantitatively than the activity of inositol triphosphate in driving calcium metabolism inside cells: Am I correct? Is this a possible explanation?

DR. CARITIS. First, I would like to respond to Dr. Cefalo's questions, whether progesterone production is affected by the administration of ritodrine and, if so, is there a difference between a pulsatile and continuous infusion. We measured progesterone but have not yet measured estrogen concentrations in these animals. There was no effect of either regimen on plasma progesterone concentrations.

Furthermore, steady-state concentrations were similar in the two groups. Thus, if there was an effect of ritodrine on the progesterone concentrations or β-adrenergic receptor density, the effect would have been comparable in the two groups.

In response to the question about biopsy site, our biopsies were always done between cotyledons. A biopsy over the cotyledonary site would lead to fairly heavy bleeding so we tried to avoid performing biopsies near cotyledons.

In the previous study, where we validated our animal model, we obtained myometrial biopsy specimens from various regions to determine the regional variation of β-adrenergic receptor density. We found, on average, about a 22% regional variation. Most of the variation occurred when we compared receptor density from tissue close to the cervix to tissue distant from the cervix. To minimize the regional variation in receptor density, we obtained two biopsy specimens at each time point.

Dr. Cefalo asked if there is some way we could separate vascular and uterine smooth muscle β-adrenergic receptors. When we obtained our myometrial tissue, we scraped the tissue with a glass slide to remove decidua and the large blood vessels. Smaller blood vessels and capillaries, however, were not removed.

It is assumed that the contribution to the total β-adrenergic receptor density by vascular β-adrenergic receptors is small. Further, since each animal serves as its own control, the contribution of vascular β-adrenergic receptors to the entire receptor pool would be constant.

I'm afraid I can't answer Dr. Kirschbaum's question. There is ample evidence that cAMP is not the only regulator of smooth muscle contractility. Some of our own data do not quite fit a model that relies totally on cAMP as the overall determinant of uterine contractility.

Rapid intramembranous absorption into the fetal circulation of arginine vasopressin injected intraamniotically

William M. Gilbert, MD, Cecilia Y. Cheung, PhD, and Robert A. Brace, PhD
San Diego, California

Recently an intramembranous pathway was reported in the ovine fetus as a route for the movement of a significant volume of water from the amniotic cavity directly into the fetal blood, which perfuses the fetal membranes and fetal surface of the placenta. To test whether this pathway could be an avenue for the movement of arginine vasopressin from the amniotic cavity into the fetal circulation, we injected 1 to 25 μg of arginine vasopressin into the amniotic cavity of two groups of chronically catheterized fetal sheep: a control group of seven animals and a group of seven animals with surgical ligation of the fetal esophagus. We found similar and highly significant increases of arginine vasopressin concentrations in both control and surgically ligated fetuses in amniotic fluid ($p < 0.00001$), fetal plasma ($p < 0.0001$), and fetal urine ($p < 0.0001$). Both groups had similar increases in arterial ($p < 0.0001$) and venous ($p < 0.003$) pressures with simultaneous decreases in urine flow ($p < 0.001$) and heart rate ($p < 0.0001$) after the intraamniotic injection of arginine vasopressin. We conclude that amniotic arginine vasopressin can be rapidly absorbed in its biologically active form directly into the fetal circulation through the intramembranous pathway. Furthermore, the observation that esophageal ligation did not alter this absorption suggests that the intramembranous pathway may be important in the regulation of amniotic fluid volume and composition. (Am J Obstet Gynecol 1991;164:1013-20.)

Key words: Vasopressin, amniotic fluid, intramembranous pathway

The intramembranous pathway for the movement of water from the amniotic cavity directly into the fetal blood, which perfuses the fetal membranes and fetal surface of the placenta, has been described in pregnant sheep.[1] In our previous study, we found that warmed distilled water injected into the amniotic cavity was rapidly absorbed into the fetal circulation, resulting in highly significant decreases in plasma osmolalities and electrolyte concentrations. Furthermore, this absorption of amniotic water occurred more rapidly in a group of fetuses with ligated esophagi. These observations clearly showed that not only can significant volumes of amniotic water be absorbed through the intramembranous pathway into the fetal circulation under normal physiologic conditions, but also that ligation of the fetal esophagus resulted in an increase in conductance of the intramembranous pathway. This raises the possibility that the intramembranous pathway may play a primary role in the determination of both the volume and the composition of the amniotic fluid.

Arginine vasopressin is normally present in fetal plasma and amniotic fluid. Arginine vasopressin is a low molecular weight (1084 daltons) vasoactive substance that has significant cardiovascular and renal effects in the fetus. Arginine vasopressin is present early in gestation and is known to increase acutely during periods of fetal stress including hypoxia and hemorrhage.[2-4] Infusions of arginine vasopressin into the fetal circulation resulted in a dose-dependent increase in arterial and venous pressures and decreases in urine output and heart rate.[5, 6]

Arginine vasopressin is found in amniotic fluid at levels comparable to those in fetal plasma and the question arises as to what overall role arginine vasopressin may play in amniotic fluid volume regulation. Amniotic fluid arginine vasopressin may act as a reservoir for fetal plasma arginine vasopressin because injections of arginine vasopressin into the amniotic cavity resulted in sustained high amniotic levels for 24 hours or longer.[7] Previously, Ervin et al.[8] injected 25 μg of arginine vasopressin into the amniotic cavity of fetal sheep to examine the recirculation of arginine vasopressin from the amniotic fluid into the fetus. Their study showed significant increases in fetal plasma arginine vasopressin concentrations within 30 minutes after the injection without significant changes in fetal blood pressure or heart rate. They concluded that arginine vasopressin was absorbed by fetal swallowing.

To determine whether the intramembranous pathway can provide a means for recirculation of amniotic

From the Division of Perinatal Medicine, Department of Reproductive Medicine, University of California at San Diego.
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Reprint requests: William M. Gilbert, MD, 225 Dickinson St., H-813, San Diego, CA 92103.
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fluid arginine vasopressin in addition to fetal swallowing, this study was designed to examine whether this pathway could play a role in the movement of arginine vasopressin from the amniotic cavity into the fetal circulation in intact and esophageal-ligated fetuses.

Methods

The protocol for this experiment was approved by the animal use committee in our university and we followed the National Institutes of Health guidelines for the use and care of laboratory animals. Fourteen time-dated pregnant sheep (Nebeker Ranch, Lancaster, Calif.) at a mean (±SEM) of 128 ± 1 days' gestation at the time of surgery were used for this study. The surgical procedures, briefly described here, are detailed elsewhere.[9] The animals were anesthetized with 1 gm of thiamylal sodium given intravenously, and maintained with halothane (0.5% to 2%) in oxygen. In the supine position, using aseptic techniques, a midline incision was made in the abdominal wall and the uterus was exposed through the abdominal incision. The fetal hind limbs were brought out of a uterine incision and bilateral anterior pedal arterial and saphenous venous catheters were placed and advanced to the level of the diaphragm. A suprapubic bladder catheter was placed through a lower abdominal incision. Amniotic fluid catheters were tied to each hind leg and bladder catheter incision site. The fetus was returned to the uterus and fetal membranes were ligated around the catheters twice to prevent leakage of amniotic fluid. The uterus was closed in a watertight manner with a continuous suture. In seven of the animals a second uterine incision was made over the fetal neck and the esophagus was identified and ligated twice. The incision in the neck was closed as was the uterine incision in a similar watertight manner as described in this article. Maternal femoral arterial and venous catheters were placed and advanced to the level of the maternal diaphragm. All catheters were tunneled subcutaneously into a pouch sewn on the flank of the ewe.

The vascular catheters were flushed daily with heparin sodium (1000 U/ml). Antibiotics (1 gm ampicillin, Omnipen, Wyeth Laboratories, Philadelphia) were given daily into the amniotic cavity and intramuscularly (800,000 U of penicillin and 1 gm dihydrostreptomycin) to the ewe during the postoperative period.

Experiments were begun 5 days after surgery. The fetuses were monitored continuously for arterial and venous pressure levels, amniotic fluid pressure, urine flow rate, and heart rate. After a 30-minute control period, a bolus of arginine vasopressin (1 to 25 μg) was injected into the amniotic cavity of seven control fetuses and seven esophageal-ligated fetuses. Immediately after injection of the arginine vasopressin, the amniotic fluid was mixed by repeated withdrawal and reinjection of 60 ml of amniotic fluid. Fetal and maternal plasma, amniotic fluid, and fetal urine were sampled at 10 and 20 minutes during the 30-minute control period, and at 10, 20, 30, 45, 60, and 120 minutes after the injection of arginine vasopressin. Fetal arterial blood was sampled for blood gas and pH values (model 1302 blood gas analyzer; Instrumentation Laboratory, Lexington, Mass.), sodium, chloride, and potassium ion concentrations (Nova model 5 + 5 electrolyte analyzer), osmolality (freezing-point depression, Advanced Instruments Inc., Needham Heights, Mass.), hematocrit, and plasma concentration of arginine vasopressin. Maternal blood, amniotic fluid, and fetal urine were sampled for electrolytes, osmolality, and plasma arginine vasopressin concentration at the same times as above. Fetal urine was collected from the bladder catheter, which first went through a computer-controlled roller pump. There was approximately 5 ml of catheter dead space between the bladder and sampling port. With a basal urine flow rate of 0.4 ml/min, urine samples were obtained with a 15-minute delay relative to the respective plasma and amniotic samples. Fetal urinary bladder catheters were functioning in six control animals and five surgically ligated animals at the time of experimentation.

Radioimmunoassay. Plasma, urine, and amniotic samples were extracted with C18 microcolumns (Sep-Pak, Waters Associates, Milford, Mass.) and arginine vasopressin concentrations were measured by radioimmunoassay with an antiserum generously provided by Dr. Ben U (University of California, San Diego) and tracer obtained from New England Nuclear (Boston) as previously described.[10] Extraction recovery averaged 84 ± 3% and sensitivity was 0.2 pg/tube. The intraassay and interassay coefficients of variation were 5.6% and 16%, respectively.

Statistical analyses. The data are presented as the mean ± SE. Changes with time in a single variable were analyzed with a two-factor analysis of variance with time and animal being the two factors. All arginine vasopressin concentrations (urine, plasma, and amniotic fluid samples) were log transformed before analysis to correct for skewness of data. To determine whether changes in time in one variable were significantly different in the two groups of animals, a three-factor analysis of variance was used with time, treatment, and animal being the factors. Statistical significance was accepted at $p \leq 0.05$ and changes referred to as significant are at this level unless specified otherwise.

At the end of the experiment, the animal was killed with sodium pentobarbital (300 mg/kg), an autopsy was performed on the fetus, and fetal weight was recorded.

Results

Mean values during the control period for the two groups of animals are shown in Table I and are not different from those values found previously in our

Table I. Mean (±SE) control values

	Control	*Ligated esophagus*
Fetus		
Gestational age (days)*	133 ± 1	132 ± 1
Urine flow rate (ml/min)†	0.47 ± 0.08	0.34 ± 0.01
pH	7.34 ± 0.01	7.35 ± 0.01
P_{CO_2} (torr)	52.3 ± 1.0	52.1 ± 1.7
P_{O_2} (torr)	21.3 ± 0.7	20.7 ± 1.7
Osmolality (μOsm)		
Blood	298 ± 1	301 ± 2
Urine†	164 ± 30	175 ± 14
Amniotic fluid	275 ± 5	283 ± 3
Hematocrit (%)	31.0 ± 1.5	32.8 ± 1.5
Weight (kg)	3.6 ± 0.1	3.1 ± 0.4
Vasopressin (ng/ml)		
Fetal plasma	4.8 ± 1.7	5.1 ± 1.0
Urine	8.5 ± 2.8	6.4 ± 0.8
Amniotic	4.0 ± 0.3	5.6 ± 1.0
Maternal plasma	3.1 ± 0.6	3.4 ± 1.0
Mother		
Blood osmolality	298 ± 3	301 ± 2

Data from seven control and seven surgically ligated fetuses.

*Gestational age at start of experiment.

†Urine samples from six control and five surgically ligated fetuses.

laboratory. Fig. 1 shows the arginine vasopressin concentrations in the amniotic fluid before and after the injection of 25 μg arginine vasopressin into the amniotic cavity in the two groups of fetuses. Although the changes with time were highly significant ($p < 0.00001$) there was no difference between the control and ligated animals in amniotic fluid concentrations of arginine vasopressin at any time throughout the experiment.

The fetal plasma arginine vasopressin concentrations are presented in Fig. 2. Significant increases ($p < 0.00001$) were observed in both groups as early as 10 minutes after arginine vasopressin injection. Although plasma arginine vasopressin concentrations in the fetuses with esophageal ligation were on average higher than those in the control fetuses, there was no statistically significant difference between the two groups.

Fig. 3 shows the changes in fetal arterial pressure, venous pressure, and heart rate after the injection of arginine vasopressin. Significant increases occurred in arterial pressure ($p < 0.00001$) and there were no significant differences between groups. When the two groups were analyzed individually, arterial pressure increased significantly at 10 minutes in the ligated group and at 20 minutes in the control group. The venous pressure increased ($p < 0.003$) and showed no intergroup differences. The fetal heart rate decreased significantly ($p < 0.00001$) with changes occurring at 15 minutes in the ligated group and at 20 minutes in the control group. These heart rate changes were similar between the two groups.

In the animals from which urine was available, urine

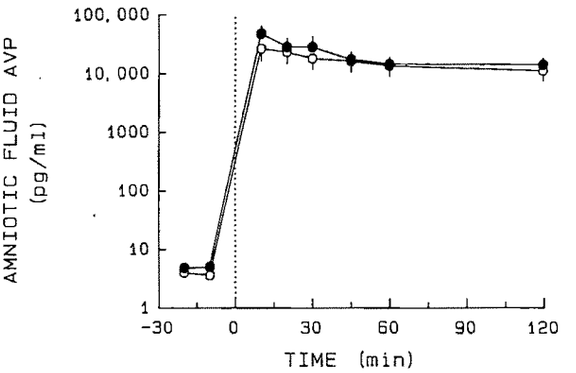


Fig. 1. Mean (±SEM) amniotic fluid arginine vasopressin *(AVP)* concentrations (log scale) after injection of 1 to 25 μg of arginine vasopressin into the amniotic cavity *(dotted vertical line)*. Changes from the control period are highly significant ($p < 0.00001$) for both the control and surgically ligated groups. *Closed circles* represent surgically ligated fetuses, *open circles* represent control animals.

arginine vasopressin concentrations (Fig. 4) increased significantly ($p < 0.0001$) with no difference between the control and ligated groups. The rise occurred within 45 minutes in both groups. Urine osmolality, sodium ion, and chloride ion increased (Fig. 5) concurrently with the increases in urine arginine vasopressin concentrations. Urine osmolality increased significantly ($p < 0.0001$) and the rise occurred in both groups by 45 minutes. Urine sodium and chloride ion concentrations increased significantly ($p < 0.0001$) at 45 minutes in both groups with no intergroup differences. Fetal urine flow rate (Fig. 6) decreased ($p < 0.001$) similarly in both groups of fetuses and there was no difference between the two groups.

The fetal plasma pH decreased significantly in both groups at two hours after the injection ($p < 0.01$), and there was no difference between groups. The fetal plasma P_{CO_2} values increased significantly by 1 mm Hg at 2 hours after the injection ($p < 0.05$) and there was no difference between the two groups. The fetal pO_2 value did not change in either group. There were no significant changes in any of the other fetal or maternal parameters after the injection of arginine vasopressin.

The amniotic fluid osmolality increased ($p < 0.001$) similarly and significantly in both groups after the arginine vasopressin injection. This occurred simultaneously with the increase in urine osmolality and arginine vasopressin concentration. The amniotic sodium and chloride ion concentrations increased significantly ($p = 0.05$ for sodium, $p < 0.01$ for chloride) by the second hour after the injection in parallel with amniotic osmolality with no difference between groups.

Comment

In this study we showed that arginine vasopressin (molecular weight of 1084 daltons) can rapidly enter

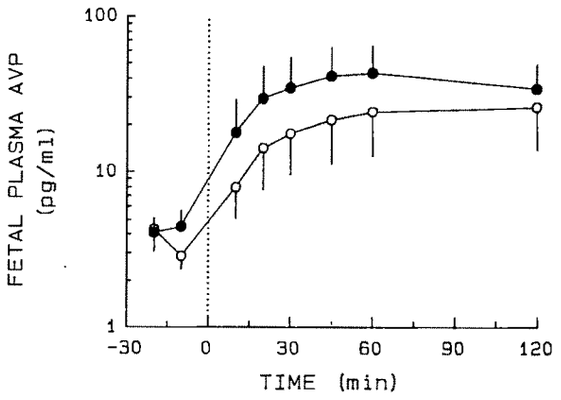


Fig. 2. Mean (±SEM) fetal plasma arginine vasopressin *(AVP)* concentration (log scale) after injection of 1 to 25 μg of arginine vasopressin *(dotted vertical line)* into the amniotic cavity. Both groups (*closed circles* represent surgically ligated, *open circles* represent control) had significant increases ($p < 0.0001$, ligated; $p < 0.002$, control) and were not different from each other.

the fetal circulation through the intramembranous pathway independent of fetal swallowing. Furthermore, this absorbed arginine vasopressin has physiologic activity as manifested by pronounced effects on both the cardiovascular and renal systems. Finally, these observations suggest that the intramembranous pathway may be the major route for uptake of arginine vasopressin from the amniotic cavity into the fetal circulation.

Arginine vasopressin secretion is generally enhanced during fetal stress. In addition to its cardiovascular actions, arginine vasopressin acts on the fetal kidney to increase the reabsorption of water and cause excretion of a more concentrated urine. During the time when plasma arginine vasopressin levels are elevated, urine contains high levels of arginine vasopressin, which enters the amniotic cavity, and amniotic arginine vasopressin concentration increases. The levels of amniotic arginine vasopressin remain elevated for periods of up to 24 hours after acute hypoxia or arginine vasopressin injection into the amniotic cavity of fetal sheep.[7] To what degree arginine vasopressin normally exchanges directly between fetal blood and the amniotic fluid is unknown. However, in three of six control fetuses during the preinjection period in our study, fetal urine arginine vasopressin concentration was the highest with fetal plasma lowest and amniotic fluid arginine vasopressin concentration intermediate. If amniotic fluid arginine vasopressin was removed only by swallowing, one may expect that amniotic fluid arginine vasopressin would equal urine arginine vasopressin under basal conditions. With a lower amniotic fluid arginine vasopressin concentration than that of urine, arginine vasopressin appears to leave the amniotic compartment by some route other than swallowing. This is further supported by the observation that in three of five ligated fetuses under basal conditions, the same urine-amniotic-plasma concentration profile existed as above. This, of course, assumes that enzymatic degradation of amniotic arginine vasopressin was slight as suggested by persistent elevations in amniotic arginine vasopressin in other studies in sheep.[7]

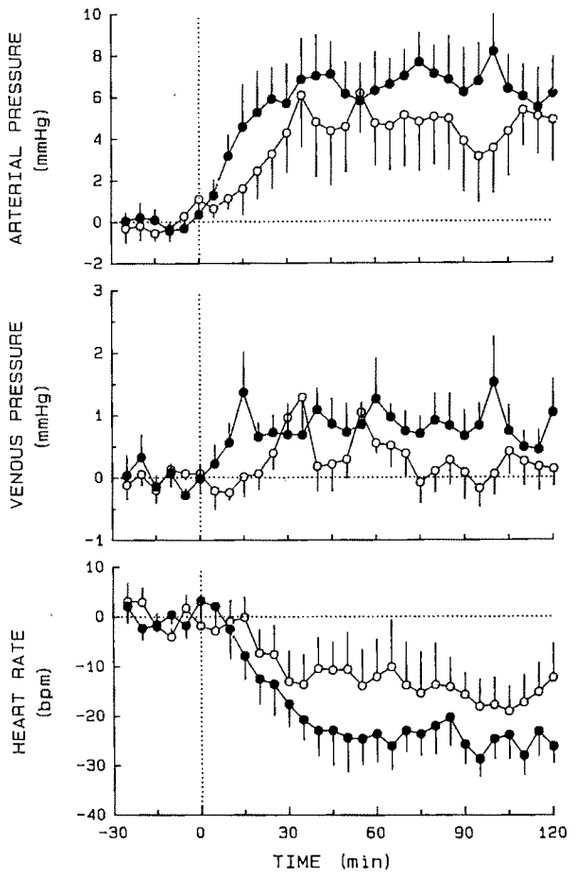


Fig. 3. Mean (±SEM) change from control in fetal arterial pressure *(upper)*, venous pressure *(middle)*, and heart rate *(lower)*. *Closed circles* represent the surgically ligated fetuses, *open circles* represent control fetuses. Arterial pressure increased significantly in both groups ($p < 0.00001$, ligated; $p < 0.002$, control) and were not different from each other. Venous pressures increased significantly ($p < 0.0001$, ligated; $p < 0.003$, control) and were not different from each other. Fetal heart rate decreased significantly in both groups ($p < 0.00001$, ligated; $p < 0.02$, control) and were not different from each other. *Dotted vertical line* is time of injection.

In our earlier study[1] we found that the conductance of the intramembranous pathway to amniotic water was increased with ligation of the fetal esophagus and we believed that this could possibly explain the finding of occasionally normal amniotic fluid volumes in the absence of fetal swallowing.[11, 12] This study is not inconsistent with the concept of an increase in conductance of the intramembranous pathway to amniotic arginine vasopressin after esophageal ligation because in every measured parameter (Figs. 1 to 6) the ligated animals had a larger response than that of the control animals.

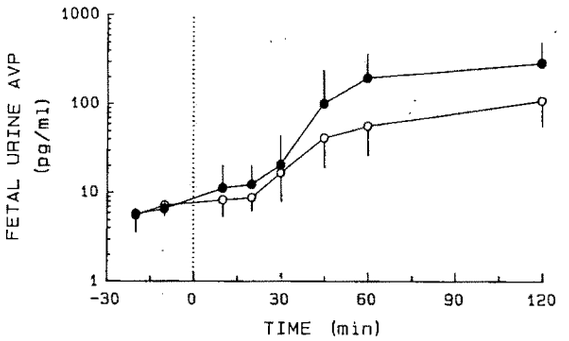


Fig. 4. Mean (±SEM) fetal urine arginine vasopressin *(AVP)* concentration (log scale) after injection of 1 to 25 μg *(dotted vertical line)* into the amniotic cavity. Both groups had significant increases ($p < 0.0001$, control; $p < 0.0001$, ligated) and were not different from each other. *Closed circles* represent surgically ligated fetuses, *open circles* represent control fetuses.

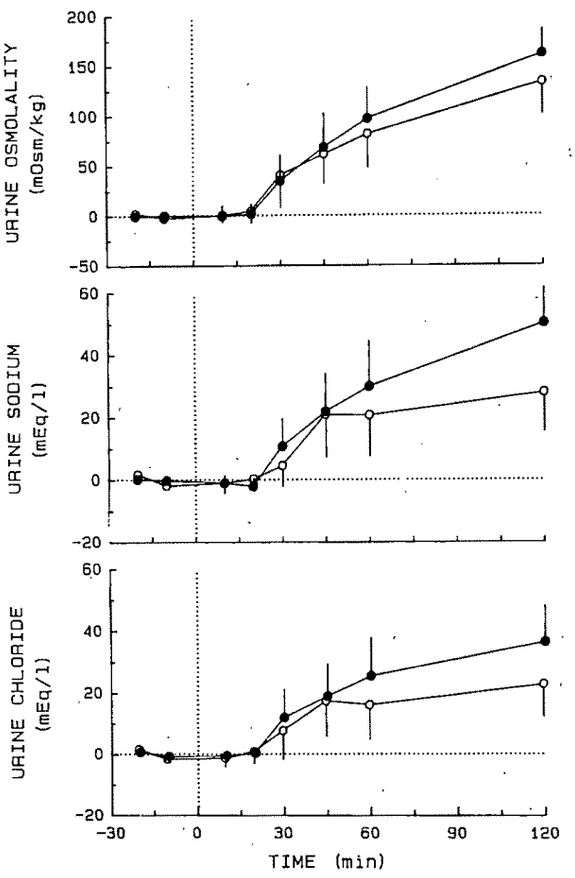


Fig. 5. Mean (±SEM) change in fetal urine osmolality and sodium and chloride ion concentrations after injection of arginine vasopressin *(dotted vertical line)* into the amniotic cavity. Both groups showed significant increases in urine osmolalities after injection of arginine vasopressin and were not different from each other. *Closed circles* represent surgically ligated ($p < 0.0001$), *open circles* represent control ($p < 0.001$). Both groups of animals showed significant increases in urine sodium ($p < 0.0001$) and urine chloride ($p < 0.0001$) and were not different from each other.

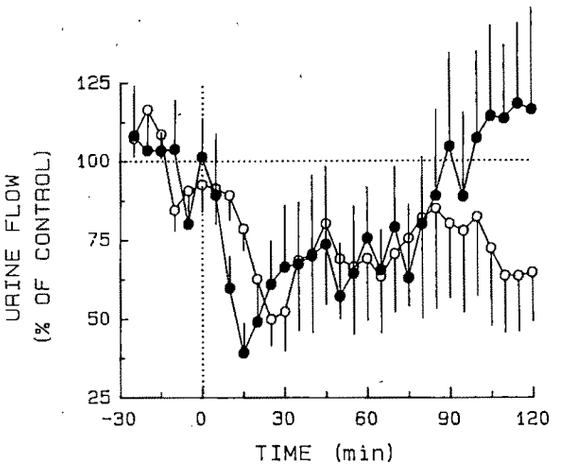


Fig. 6. Mean (±SEM) change from control urine flow after injection of arginine vasopressin *(dotted vertical line)*. Both groups had significant decreases in urine flow ($p < 0.001$) and were not different from each other. *Closed circles* represent ligated fetuses, *open circles* represent control fetuses.

Individually there were no statistically significant differences between the groups, but collectively there was a tendency for a greater response in the ligated animals.

We used the same experimental protocol as Ervin et al.[7] and found similar significant increases in fetal plasma and urine arginine vasopressin concentrations as well as increases in urine osmolality and urinary sodium. In contrast to their finding of no changes, we found highly significant increases in fetal arterial pressure and decreases in heart rate and urine flow. However, their Table I showed nonsignificant changes in these variables in the same direction as in this study. Presumably the difference in statistical significance is a result of better resolution of the data with our on-line data acquisition system. However, in contrast to their conclusion that amniotic arginine vasopressin was absorbed by fetal swallowing, we observed essentially identical changes in fetuses with ligated esophagi. Because swallowing could not have been the cause of the increase in fetal plasma arginine vasopressin concentration in the ligated animals, it appears that the intramembranous pathway may be the primary route for amniotic arginine vasopressin to enter the fetal circulation.

The fetal cardiovascular responses obtained in this study are similar to those found during intravascular infusions of arginine vasopressin when comparable plasma concentrations of arginine vasopressin were achieved.[5, 13] Thus the absorbed arginine vasopressin retained its biologic activity. In comparison, the gastrointestinal system might be expected to degrade swallowed arginine vasopressin.

Our results showed that the changes in all urinary parameters except urine flow were delayed in relation

to the cardiovascular and plasma arginine vasopressin concentrations. This was due to a delay in collection of urine samples that passed through the tubing and a roller pump before sampling. Furthermore, there is a natural delay in the urine collection that must leave the kidney and enter the bladder before being sampled. Thus the urine changes may have occurred simultaneously with changes in the other variables. In Fig. 6 the urine flow in the ligated group returns to control values at 90 minutes after the injection into the amniotic cavity, whereas the control urine flow stays depressed. This finding is consistent with findings of Lingwood et al.[6] who reported a decrease in urine flow at low doses of arginine vasopressin infused into the fetal circulation, but an increase in urine flow at higher dosages. They believed that the increase in urine flow was a result of an arginine vasopressin presser effect that overrides the renal effect.

Collectively, the previous study and this study suggest that the intramembranous pathway may be the primary route by which amniotic fluid arginine vasopressin reenters the fetal circulation. The assumption that arginine vasopressin is representative of similar or smaller molecular weight substances suggests that the intramembranous pathway may play a major role in the regulation of amniotic fluid volume and composition.

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Discussion

DR. ROBERT CREASY, Houston, Texas. Amniotic fluid regulation has been a source of many questions, investigations, and theories for decades. This current investigation studies the movement of biologically active arginine vasopressin from the amniotic cavity of the chronically instrumented pregnant ewe. One half the animals studied also had the fetal esophagus doubly ligated to interfere with fetal swallowing of amniotic fluid and the uptake of its components, including the exogenously derived increase of arginine vasopressin. I presume that at autopsy the authors confirmed that the esophagi of these fetuses were not patent. The results appear to clearly show that the arginine vasopressin injected into the amniotic cavity quickly (within 10 minutes) resulted in an increase in fetal plasma arginine vasopressin. This increase in fetal plasma arginine vasopressin was then associated with a number of known biologic responses within the fetus. There were no detectable differences noted between the fetuses with ligated esophagi compared with those not ligated. However, it is interesting to note that there were consistent trends to greater responses in fetal plasma arginine vasopressin, arterial and venous pressure, heart rate, urine arginine vasopressin, urine osmolality, and urine sodium in the ligated fetuses. Perhaps the authors could speculate on why such pronounced consistent trends for greater responses were present in the esophageal ligation group. Also noteworthy is the return to normal in urine flow at 90 to 120 minutes in the esophageal ligation group, a feature not present in the nonesophageal ligation group. These experiments and previous ones by these authors indicate that not only water but other components of amniotic fluid may be rapidly absorbed by a intramembranous pathway into the fetal circulation in the ewe and have biologic activity at the gestational age studied.

The increase in arginine vasopressin in fetal plasma results in an increase in urinary arginine vasopressin and a continuing increase in amniotic fluid arginine vasopressin. Previous experiments indicate the amniotic fluid arginine vasopressin concentrations may remain elevated for 24 hours. Presumably, this arginine vasopressin may recirculate again into the fetal circulation via the transmembranous pathway and remain biologically active inasmuch as all their experimental variables remain altered at 120 minutes except for one, namely urinary flow. The curious result is that urinary flow returned to normal by 90 minutes in fetuses with esophageal ligation, yet urinary flow in the control

group remained depressed. I would appreciate if the authors could theorize why urinary flow is normal after 90 minutes in the group of fetuses with esophageal ligations when all other experimental variables in both groups remain changed at up to 120 minutes. How long does arginine vasopressin remain biologically active and in what compartments?

A small point that needs to be addressed is the radioimmunoassay for arginine vasopressin. An interassay coefficient of variation of 16% seems somewhat excessive.

Last, I wonder whether the authors believe that the intramembranous pathway is operable in human gestation? Could such a pathway involve other substances such as α-fetoprotein, steroids, and monokines.

Dr. Sheldon H. Cherry, New York City, New York. In the late 1960s we did some work at Mt. Sinai on elucidating the mechanism for bilirubin transport into the amniotic cavity in erythroblastosis. It was our thesis that bilirubin in amniotic fluid is unconjugated bilirubin and it was related to the protein content in the amniotic fluid. We could put protein (albumin) into the amniotic fluid cavity and thereby raise the bilirubin content. This we believed showed the efficacy of the intramembranous area for transport and relates to this article.

It was our thesis that the umbilical cord vessels (and the fetal surface of the placenta) were important transport sites as was mentioned in your article. I would like your opinion on this question.

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Dr. Warren M. Crosby, Oklahoma City, Oklahoma. It was not clear in the abstract or in the article whether the control animals were sham operated. If the control animals had no surgical exploration in the neck, I think the conclusion you drew might be invalid. When operating in the neck there are chemoreceptors and baroreceptors that are disturbed and that may release autonomic vasoactive substances.

Dr. R. Besinger, Maywood, Illinois. I congratulate Dr. Gilbert on a provocative study, but I have a question. We all know that fetal sheep breathe, and I am curious whether the author would like to address the issue of a potential pulmonary pathway for transmission of these substances from the amniotic fluid.

Dr. Thomas H. Kirschbaum, Los Angeles, California. Dr. Gilbert, I wonder whether you measured maternal arginine vasopressin concentration in the course of this work. I understand you have excluded the possibility of transmission from amniotic fluid to the mother and then across the placenta to the fetus on morphologic grounds dealing with the localized maternal perfusion in the sheep. But I wonder whether you checked it by following up the concentrations in the maternal blood.

Dr. Robert Barbieri, Stony Brook, New York. In human beings arginine vasopressin is well absorbed across nasal and conjunctival membranes. In fact, that is the way it is administered for therapy. I wonder whether the authors looked at the possibility that the oral membrane, conjunctive or nasal mucosa, are roots of absorption of arginine vasopressin.

Dr. John P. Gusdon, Jonesboro, Georgia. I just had a déjà vu of some work done around 30-some years ago at the University of Virginia by Dr. Harry Gaughey. You asked some of the same questions, although his work was in vitro. I would like to know what method was used in your study to measure arginine vasopressin.

Dr. Gilbert (Closing). At autopsy we documented the nonpatency of all the ligated esophagi. One point I did not address in my discussion was the tendency toward an increase in the fetal plasma arginine vasopressin concentrations as well as a change in all the parameters measured was seen in the ligated group as compared with controls.

In our original water-injection study, we found that there was an increase in absorption of water with the ligation of the fetal esophagus. When we estimated the amount of water movement, we found that under normal physiologic conditions about 200 ml per day would go from the amniotic cavity into the fetal circulation. That happened to almost exactly equal the differences found between fetal urination and fetal swallowing in multiple animal and human studies.

In our study we found that with the injection of the arginine vasopressin into the amniotic cavity we did not find a statistically significant increase in conductance of the intramembranous pathway.

However, I do believe that tendency probably is there. If an increase in water absorption is found with the ligation of the fetal esophagus, it would make sense to me that there would be an increase in arginine vasopressin absorption either by diffusion or with the bulk flow of water. So I think that finding those tendencies toward an increase is consistent with our previous water finding. That we did not have enough of a difference to make a statistical difference is interesting.

The question about the return to normal on the urine flow in the ligated group is interesting. One of the first mechanisms of arginine vasopressin action on the kidney is a decrease in urine flow, which we found. If you remember from Fig. 6, the initial drop in urine flow in the ligated group was the most significant initially.

Arginine vasopressin increases blood pressure, and in the ligated group there is a higher rate of increase in blood pressure, both arterial and venous, as compared with the control group. At some point there is an overriding effect of blood pressure on the kidneys and you have an increase in urine flow. I was not surprised that there was a return to normal and then an increase in urine flow as the pressure head on the kidney increased.

The half-life of arginine vasopressin in fetal plasma has been reported to be about 5 to 15 minutes. Few

studies have looked at the half-life in amniotic fluid in the amniotic cavity. Several authors have found that after injection into the amniotic cavity arginine vasopressin is still markedly elevated at 24 hours. There is decreased arginine vasopressinase activity present in the amniotic cavity.

With regard to the question about the human applications, even though the sheep placentation is markedly different from that of human beings in multiple ways, there are many reports in the literature of either primates or human beings of injections of substances into the amniotic cavity and these substances being found at the time of the cesarean section with elevations in cord levels that could not be explained by fetal swallowing. Fetal swallowing has been shown to occur in bouts of two to seven times a day at varying volumes.

So we believe that in human beings, even though it is a different placentation, the back side of the placenta is fairly large in utero and is exposed to multiple fetal vessels. I believe that potentially in the future pharmacologic agents such as digoxin may be used for fetal cardiac arrhythmias. Other substances such as thyroid hormone, or thyrotropin-releasing factor or steroids may be used for the lung maturation.

Dr. Cherry brought up the bilirubin transport and the umbilical cord. Many authors have looked in vitro at perfusing umbilical cords and at exposing them to radioactive-labeled substances. They found that there was absorption into the umbilical cord. I consider this as probably part of this overall pathway of absorption.

If you think about it from a physiologic viewpoint, minimal absorption across the umbilical cord occurs because you have got a cord with a fairly large distance across Wharton's jelly to the umbilical vessels, coupled with a high blood flow and minimal area of absorption. However, some in vitro studies found that the umbilical cord is a route for passage of labeled substances; and bilirubin very well could be that way.

No, Dr. Crosby, we did not do sham operations at the time of initial surgery. What we basically did was brought the fetal head out, made a vertical incision, found the esophagus, double ligated it, sewed it back up, and put it back in. We did not believe that the sham operation was needed. You are right, however, that to be a true control we would have needed to sham operate those animals.

The question about fetal breathing was brought up. We know that in utero it used to be thought that fluids went into the fetal lungs throughout fetal development. We know that with fetal breathing movements, larger volumes can move back and forth with every episode of fetal breathing. But when you look at an overall summation, a net outward movement of fluid occurs. In fetal sheep about 20% of fetal body weight per day is excreted through the fetal lungs. Absorption by the lungs is minimal if at all for the above reasons.

The question raised about the nasal route for absorption is a very good question. There was a report from San Francisco in the literature about digoxin being injected into the amniotic cavity (Hamamoto K, Iwamoto HS, Roman CM, Benet LZ, Rudolph AM. Fetal uptake of intraamniotic digoxin in sheep. Pediatr Res 1990;27:282-5). They measured umbilical vein levels of digoxin and dorsal aorta levels of digoxin. They found in 10 of the 11 animals that the level in the umbilical vein was less than the level in the dorsal aorta. They found a very rapid absorption into the fetal circulation. This higher dorsal aorta level than vein digoxin level made us think, are we completely wrong? What other route of absorption is there? Then we realized that in adults the nasal passage is a tremendous route for absorption of vasopressin as well as other substances.

In utero the fetal sheep is in a fetal position, and our catheters are attached to the legs close to the nose. This could have been a potential route of absorption.

To test this, very recently we put a surgical glove over the ovine fetal head. In five animals we injected arginine vasopressin in the amniotic cavity, and in five we injected it into the glove over the head. We found in the five with injections into the amniotic cavity the exact same thing we found here with the ligated group. In the group with the injection into the glove we found absolutely no change in the cardiovascular parameters. Plasma concentrations of arginine vasopressin have not been measured. I am comfortable that we have ruled out the nose as a possible route of absorption.

I cannot explain the findings of a level of dorsal aorta digoxin higher than that in the umbilical vein.

There was a question about the radioimmunoassay. Dr. Creasy had a question about coefficient of variation being 16%. The radioimmunoassay for this assay I found to be fairly consistent with that in the literature when you measure arginine vasopressin. The intraassay variation is about 4% to 5% and the interassay variation was 16% as you reported.

With regard to an earlier question, there were no differences or changes in maternal levels of arginine vasopressin measured.

Effect of intravenous cocaine on uterine blood flow in the gravid baboon

Mark A. Morgan, MD,[a] Susan L. Silavin, PhD,[a] Mildred Randolph, DVM,[b] Gerald G. Payne, Jr., MD,[a] Roger E. Sheldon, MD,[c] John I. Fishburne, Jr., MD,[a] Richard A. Wentworth, PhD,[d] and Peter W. Nathanielsz, MD, PhD[d]

Oklahoma City, Oklahoma, and Ithaca, New York

Cocaine abuse during pregnancy is associated with adverse perinatal outcome, believed to be a result of potent vasoconstrictive effects that cocaine has on the maternal cardiovascular system. The direct effect of cocaine on the pregnant, nonhuman primates' uterine vasculature in vivo has not been examined. We investigated the effects of intravenous cocaine on maternal arterial blood pressure, uterine blood flow, and uterine vascular resistance in four unanesthetized, chronically instrumented pregnant baboons. Baboons were instrumented during the latter half of pregnancy, placing an ultrasonic flow probe around one uterine artery and cathethers in the maternal inferior vena cava and aorta. Bolus intravenous cocaine hydrochloride doses of 0.05 mg/kg, 0.1 mg/kg, and 0.3 mg/kg by maternal weight were infused 30 minutes apart at least 5 days after surgical instrumentation. The maternal blood pressure rose 7.3% and 12.0% after the 0.1 mg/kg and 0.3 mg/kg cocaine infusions, respectively, and the uterine blood flow fell in a dose-dependent fashion 13.1% and 22.7%. Plasma norepinephrine levels rose in response to the 0.3 mg/kg cocaine infusion. These studies show that low doses of cocaine significantly reduce uterine blood flow in the pregnant baboon in a dose-dependent manner by increasing uterine vascular resistance. (Am J Obstet Gyneco. 1991;164:1021-30.)

Key words: Cocaine, uterine blood flow, pregnancy, baboon

Cocaine hydrochloride, as an illicit drug, is abused by 8% or more gravid women.[1] As a local anesthetic, it blocks the presynaptic reuptake of norepinephrine and dopamine, allowing these neurotransmitters to accumulate in the postsynaptic cleft and in the systemic circulation.[2-4] Thus the sympathetic nervous system is stimulated, resulting in systemic vasoconstriction, tachycardia, ventricular arrhythmias, and seizures.[5] The adverse perinatal outcomes observed in gravid women who abuse cocaine include intrauterine fetal growth retardation, congenital anomalies, preterm labor, abruptio placentae, and spontaneous subarachnoid hemorrhage are believed to be a result of profound systemic vasoconstriction.[1, 6-10] Recently this belief has been supported by investigators who observed a dose-dependent reduction of uterine blood flow in response to intravenous cocaine administered to the gravid ewe.[11-12] Although these investigations have significantly contributed to our understanding of its effect on the ewe's maternal-fetal unit, cocaine's effect in vivo on the unanesthetized, chronically instrumented, gravid nonhuman primate has not been previously reported. The purpose of this study was to determine cocaine's effect on hemodynamic parameters in the unanesthetized, chronically instrumented, gravid baboon in the third trimester of pregnancy.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology,[a] Division of Animal Resources, Department of Pathology,[b] and Division of Neonatology, Department of Pediatrics,[c] University of Oklahoma Health Sciences Center, and Laboratory for Pregnancy and Newborn Research, New York State College of Veterinary Medicine, Department of Physiology, Cornell University.[d]
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Reprint requests: Mark A. Morgan, MD, Department of Obstetrics and Gynecology, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73190.
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Material and methods

Four pregnant baboons *(Papio cynocephalus/anubis)* at 90 to 121 days' gestation (term, 175 days) from the nonhuman primate colony maintained at the University of Oklahoma Health Sciences Center were used for this study. The animals were transported from the colony site to the Biomedical Sciences Building animal care facility approximately 3 weeks before surgical instrumentation. On arrival, the animals were individually caged in sight of at least one other baboon in a lighting cycle environment of 14 hours light and 10 hours dark with lights on at midnight. Fresh water, high-protein monkey chow (Purina Mills, Inc., St. Louis), and fresh

fruit were provided daily to the baboons to eat as desired. A jacket (Alice King Chatham, Medical Arts, Los Angeles) was placed on each baboon while under light anesthesia induced by ketamine 1 week before a training tether and swivel system were attached to the jacket.

After 2 weeks of acclimation to this relatively unrestricted freedom of movement, surgical instrumentation was performed. The animals were anesthetized with intramuscular ketamine (10 mg/kg) and xylazine (0.5 mg/kg) and positioned in left lateral tilt on an operating table equipped with a warming blanket. Fetal viability was ascertained by stethoscope auscultation of the fetal heart rate. Two baboons were maintained on a continuous intravenous ketamine infusion of 0.23 to 0.28 mg/kg per minute and oxygen at a flow rate of 2 L/min administered by means of a muzzle cone. The remaining two baboons underwent endotracheal intubation and were maintained during surgery with halothane (0.75 to 1.5%) and oxygen (2 L/min) by means of an Ohio 930 Metometic anesthesia machine (Ohnedia, Madison, Wis.) Through a groin incision cathethers were placed into the aorta via a branch of the superficial femoral artery for measurement of maternal arterial blood pressure and sampling of circulating hormone concentrations, and into the inferior vena cava via a branch of the superficial femoral vein for intravenous drug administration. A muscle-splitting incision was performed in either the left or right flank to enter the pelvic retroperitoneal space. The ipsilateral external and internal iliac and uterine arteries were exposed through blunt and sharp dissection. A 2 mm pulse transit-time ultrasonic flow probe (Transonic Systems, Inc., Ithaca, N.Y.) was placed around the uterine artery. The groin and flank incisions were closed and the two vascular cathethers, and flow probe leads were tunneled subcutaneously to exit the baboon's back between the scapulae. The baboons were postoperatively maintained on a modified jacket, tether, and swivel system previously described by Stark et al.[13] This system allows for passage of the vascular catheters and flow probe leads through the roof of the cage. A box attached to the tether, anchored to the jacket on the baboon's back, contained the transducer to record arterial pressure (Cobe, Lakewood, Colo.). The vascular cathethers were flushed daily with 3 to 5 ml of 10 IU/ml heparinized saline solution.

Continuous acquisition of uterine blood flow and arterial pressure waveforms was performed with a custom-built computer-based data acquisition system. Briefly, signals are sampled at 32 Hz. The average value during 8 seconds is digitized and stored on an IBM computer with a time signal. The 32 Hz sampling frequency prevents aliasing problems with changing signals; however, the storage rate of one reading every 8 seconds allows for manageable data analysis. Details of this data acquisition system have been previously described.[14, 15]

All studies were conducted at least 5 days after surgical instrumentation. Cocaine hydrochloride (Sigma Chemical Company, St. Louis) in doses of 0.05, 0.1, and 0.3 mg/kg by maternal weight was dissolved in 5 ml of 0.9% saline solution. For each experiment the unanesthetized baboon was monitored continuously for a 45-minute baseline period before cocaine administration. During this period, arterial blood samples were taken for baseline catecholamine determinations 45, 30, and 15 minutes before cocaine administration. Each cocaine dose was administered intravenously over 30 seconds as a bolus. The 0.05 mg/kg cocaine dose was followed at 30-minute intervals by the 0.1 mg/kg and 0.3 mg/kg cocaine doses on one day. Arterial blood samples were taken for catecholamine determinations at 5 and 10 minutes after the 0.05 and 0.1 mg/kg cocaine doses and at 5, 10, 15, and 30 minutes after the 0.3 mg/kg cocaine dose. Maternal blood pressure and uterine blood flow were continuously monitored throughout the 90-minute study period.

For data analysis, maternal arterial blood pressure and uterine blood flow measurements were made 5 minutes before the initial cocaine infusion and 1, 2, 5, 10, 15, and 30 minutes after cocaine infusion. The 1- and 2-minute measurements represent all the data points over the period. Measurements at the other times represent all the data points (obtained every 8 seconds) for 1 minute before and 1 minute after the chosen time. Uterine vascular resistance was determined by the equation: UVR = MAP/UBF, where UVR = uterine vascular resistance, MAP = mean arterial pressure, and UBF = uterine blood flow. This study was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Oklahoma before initiation of the protocol.

Plasma catecholamines were measured by high-performance liquid chromatography with a 0.1 mol/L sodium acetate, 0.5 mmol/L ethylenediaminetetraacetic acid, 2.5 mmol/L hepatane sulphonic acid, and pH 5.2 mobile phase at a flow rate of 1.6 ml/min. After initial filtration and degassing, a continuous helium purge was maintained in a mobile-phase reservoir to eliminate dissolved oxygen. The reversed-phase c-18 column (Biophase ODS 25 cm, 5 μ particle size; Bioanalytical Systems Inc., West Lafayette, Ind.) was kept at 23° C. The dual electrode LC-4B amperometric detector (Bioanalytical Systems) was used at an applied potential of 625 and 675 mV in the oxidation mode with

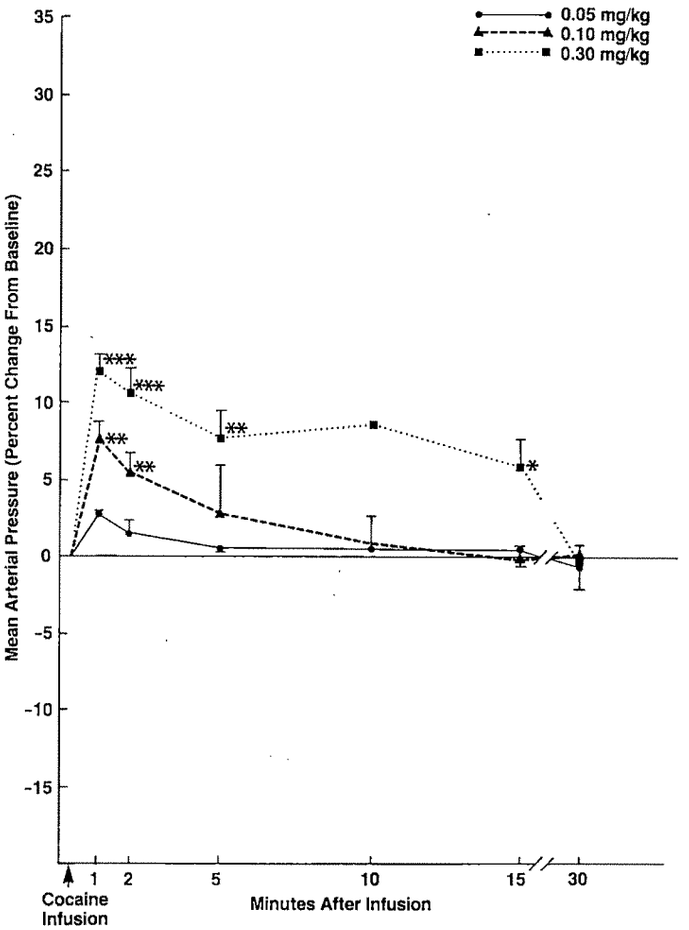


Fig. 1. Maternal mean arterial pressure responses to intravenous cocaine in the pregnant baboon at 97 to 126 days' gestation (n = 3). The following symbols represent the cocaine doses infused: ●—● 0.05 mg/kg; ▲–▲ 0.1 mg/kg; ■. . .■ 0.3 mg/kg. *Single asterisk* indicates $p < 0.05$; *two asterisks*, $p < 0.025$; and *three asterisks*, $p < 0.01$.

the gain set a 1 and 2 nA full scale deflection. A standard solution containing 500 pg of norepinephrine, epinephrine, L-dopa, dihydroxybenzylamine, and 1000 pg of dopamine and butanephrine in 200 μl of 0.1 mol/L perchloric acid was injected into every fourth sample to evaluate the chromatography conditions and for calculations. Linearity of the detection system was evaluated between 25 and 500 pg of catecholamine injected. On the basis of the signal-to-noise ratio the limit of detection was 50 pg injected for injected norepinephrine, epinephrine, and dihydroxybenzylamine. Plasma (0.5 to 1 ml) was precipitated with an equal volume of 0.6 mol/L perchloric acid, mixed and centrifuged at 2000 rpm for 10 minutes at 4° C. The supernatant was collected and stored frozen at −80° C until assayed. The acid supernatant (0.5 to 1 ml) was neutralized with 50 to 100 μl of 3 mol/L sodium hydroxide, and 25 mg of acid washed alumina (Bioanalytical Systems), 50 μl of a solution containing 625 pg of 3,4-dihydroxybenzylamine and 1 ml of 1.5 mol/L Tris hydrochloride buffer pH 8.5 were added and mixed. The extraction was carried out by shaking the mixture in a 4 ml capped conical polystyrene vial (Evergreen, Los Angeles) in a horizontal shaker for 10 minutes. After three washes with distilled water, the alumina was transferred to a filter tube assembly (Spinx Costar, Cambridge, Mass.) and centrifuged at 12,000 rpm for 2 minutes. The filter containing the dry alumina was washed with 250 μl of 0.1 mol/L perchloric acid. The perchloric acid containing the catecholamines was separated by centrifugation at 12,000 rpm for 2 minutes and transferred to an injection vial fitted with a small-sample insert (Waters Intelligent Sample Processor, Waters Associates, Milford, Mass.). The inter-

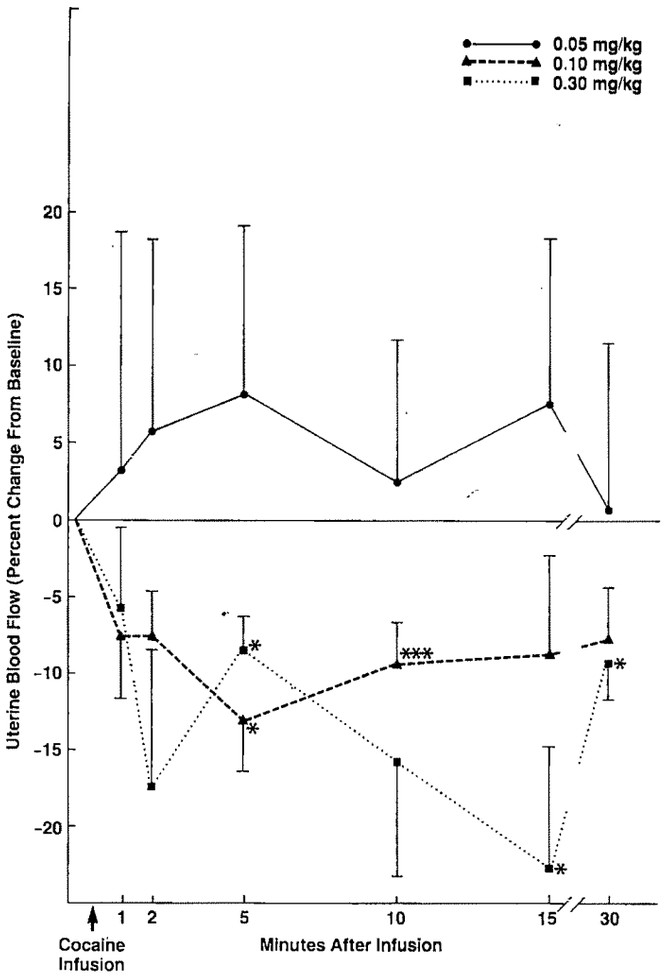


Fig. 2. Responses of uterine blood flow in intravenous cocaine in the pregnant baboon at 97 to 126 days' gestation ($n = 4$). The following symbols represent the cocaine doses infused: ●—● 0.05 mg/kg; ▲–▲ 0.1 mg/kg; ■. . .■ 0.3 mg/kg. *Single asterisk* indicates $p < 0.05$; *three asterisks*, $p < 0.01$.

assay variability was 6.5% and 10% for norepinephrine and epinephrine, respectively.

Data are reported as mean ± SEM. Statistical analysis was performed by the paired Student t test and the two-tailed independent Student t test where appropriate. Significance was set at $p < 0.05$.

Results

The mean ± SD gestational age of the pregnant baboons at the time of cocaine infusion was 115 ± 14 days. The effects of intravenous cocaine infusions on mean arterial pressure are illustrated in Fig. 1. The 0.05 mg/kg dose had no effect on the baboons' arterial blood pressure whereas the 0.1 and 0.3 mg/kg doses both resulted in significant elevations of the baboons' arterial blood pressure. The degree and duration of arterial blood pressure elevation were dose-dependent (Fig. 1).

The effects of intravenous cocaine on uterine blood flow are illustrated in Fig. 2. No significant change was observed in the uterine blood flow after the 0.05 mg/kg dose. However, significant dose-dependent reductions in uterine blood flow were observed in response to both the 0.1 and 0.3 mg/kg doses (Fig. 2). The maximal reduction of uterine blood flow appears to be dose-dependent, it was 13.1% 5 minutes after the 0.1 mg/kg dose, and 22.7% 15 minutes after the 0.3 mg/kg dose.

Uterine vascular resistance was calculated for each cocaine dose infused (Fig. 3). As previously observed with the mean arterial pressure and uterine blood flow, no significant change in uterine vascular resistance was

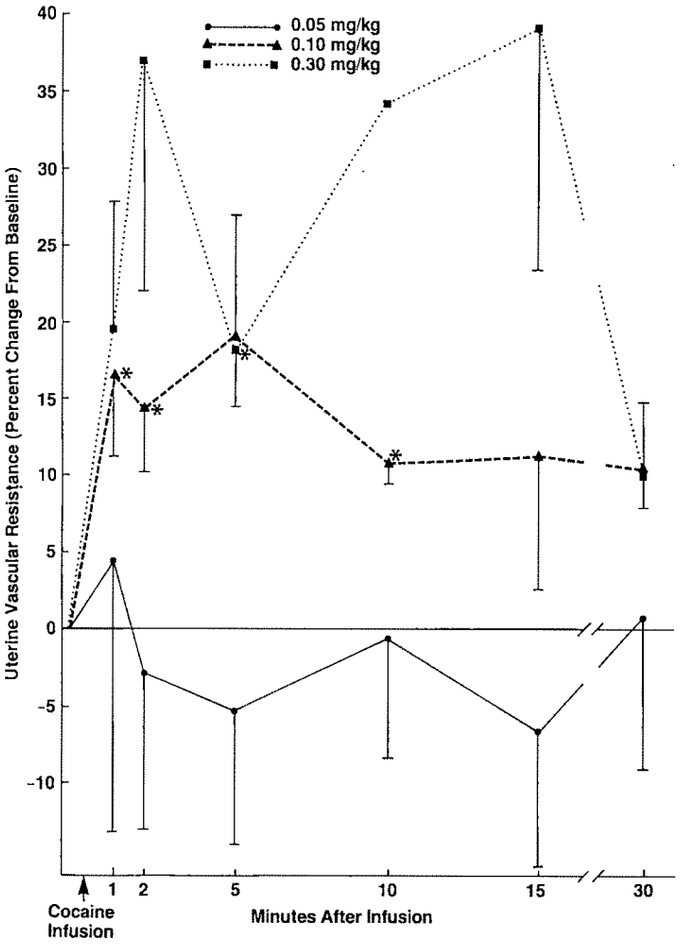


Fig. 3. Responses of uterine vascular resistance to intravenous cocaine in the pregnant baboon at 97 to 126 days' gestation (n = 3). The following symbols represent the cocaine doses infused: ●—● 0.05 mg/kg; ▲–▲ 0.1 mg/kg; ■. . .■ 0.3 mg/kg. *Single asterisk* indicates $p < 0.05$.

observed after the 0.05 mg/kg dose infusion. A significant increase in uterine vascular resistance was observed after the 0.1 and 0.3 mg/kg dose infusions. The increased uterine vascular resistance appeared to be dose-dependent as observed by the mean arterial pressure and uterine blood flow response to cocaine with a maximal increase of 10.8% 5 minutes after the 0.1 mg/kg, and 39.0% 15 minutes after the 0.3 mg/kg dose was infused.

The responses of the pregnant baboons' arterial blood pressure, uterine blood flow, and uterine vascular resistance to intravenous cocaine appears to be at least partially reflected in increases in the systemic circulation of norepinephrine (Fig. 4). Although norepinephrine levels did not change after the 0.05 and 0.1 mg/kg cocaine infusions, the 0.3 mg/kg dose resulted in a 64% increase in the systemic norepinephrine level 15 minutes after the cocaine infusion. It is interesting that the observed increase in the baboons' arterial blood pressure and the fall in uterine blood flow after the 0.1 mg/kg cocaine infusion was without an increase in the systemic norepinephrine levels. The plasma epinephrine levels remained relatively unchanged from baseline levels after each cocaine infusion. However, none of these catecholamine levels achieved statistical significance.

Comment

Cocaine, administered intravenously to pregnant baboons, produces a dose-dependent rise in arterial blood pressure and uterine vascular resistance and a dose-dependent fall in uterine blood flow. These results show a similar dose-response to intravenous cocaine as has been observed in the pregnant ewe.[11, 12] In our

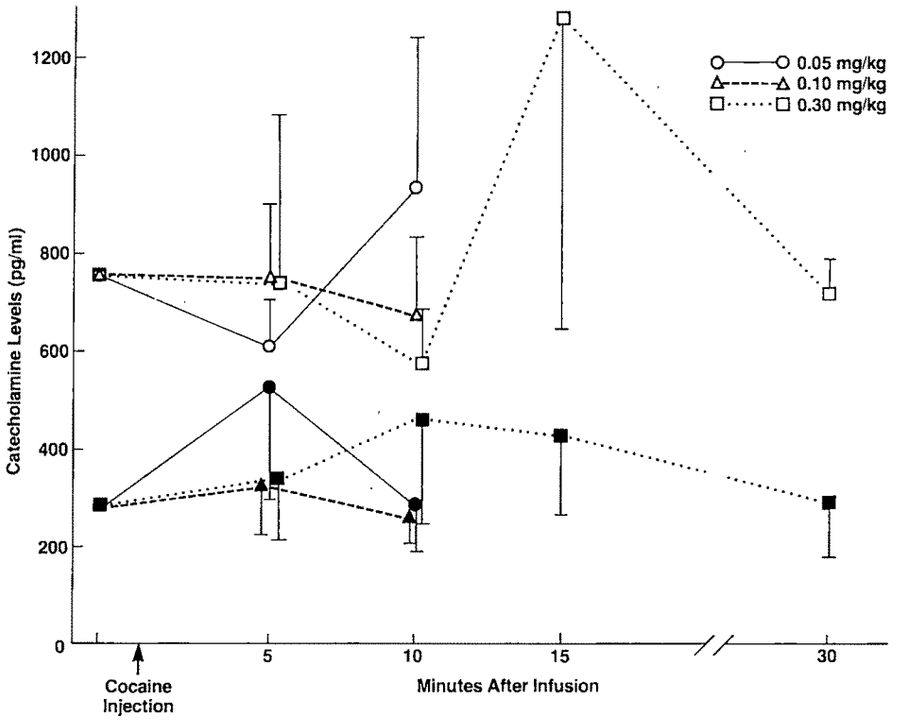


Fig. 4. Maternal catecholamine levels (norepinephrine, *open symbols;* epinephrine, *solid symbols*) in response to intravenous cocaine in the pregnant baboon at 97 to 126 days' gestation (n = 4). The following symbols represent the cocaine doses infused: ●—● 0.05 mg/kg; ▲–▲ 0.1 mg/kg; ■. . .■ 0.3 mg/kg.

study we used lower doses of cocaine than those that were administered in the studies with pregnant sheep.

The lowest cocaine dose infused into the maternal ewe by Moore et al.[11] was 0.3 mg/kg. This dose produced a 12% rise over baseline in maternal arterial blood pressure and a 30.4% fall from baseline in uterine blood flow. We similarly observed a peak rise of 12% in maternal baboon arterial blood pressure to occur 1 minute after the cocaine infusion (Fig. 1). The fall in uterine blood flow in the pregnant baboon, however, was not as marked as that observed in the maternal ewe (22.7% versus 30.4%). This small difference of uterine blood flow reduction is probably not significant but may be related to differences either in species or methods between the two investigations.

Although cocaine blocks the reuptake of norepinephrine at the presynaptic nerve terminal,[2] the relationship between the observed cardiovascular changes and systemic norepinephrine levels in response to cocaine administration have not been clearly elucidated.[17,18] Moore et al.[11] observed a doubling of systemic norepinephrine levels in response to a 0.5 mg/kg cocaine infusion in the maternal ewe; however, we observed a 64% increase in systemic norepinephrine levels in response to a lower cocaine dose (0.3 mg/kg). Our lack of a statistically significant increase in the norepinephrine level observed 15 minutes after the 0.3 mg/kg cocaine infusion could be a result of the small number of animals or a differing threshold effect in individual animals.

Even though a significant increase in maternal arterial blood pressure and fall in uterine blood flow was observed in the baboon after the 0.1 mg/kg dose, changes in systemically measured norepinephrine levels were not shown. This observation in the baboon may be related to either the absence of blood samples immediately after the cocaine administration or the failure of norepinephrine concentrations within the tissues to be reflected in the systemic circulation at this dose.

Local cocaine blockade of norepinephrine's reuptake may be enough to elicit vasoconstrictive action in uterine vasculature without any rise in systemic norepinephrine levels as we observed after the 0.1 mg/kg cocaine infusion. This presumed local effect of cocaine seems to be supported by the observations of Dolkart et al.[18] They showed in the pregnant ewe that α_1-

adrenergic receptor blockade with phenoxybenzamine hydrochloride ablated the maternal arterial blood pressure response to cocaine's administration, whereas the effect of cocaine on uterine blood flow was unchanged. In contrast, Moore et al.[11] reported that pretreatment with phentolamine (a partial α_1-adrenergic receptor blocker) did not change either the maternal blood pressure or uterine vascular response to infusion of cocaine. They speculated that cocaine may exert its vasoconstrictive effect through a mechanism that does not involve the α-adrenergic receptor. However, norepinephrine levels were not measured after both α_1-adrenergic receptor blockade and cocaine infusion.

In view of the differences in placentation and uterine blood flow in ruminants and primates it is important to obtain data in experimental situations from nonhuman primate experimental animal models. Our study illustrates that even low doses of intravenous cocaine administered to a primate species results in vasoconstrictive cardiovascular changes. Although there are reported physiological and placental differences between human and baboon pregnancies,[19,20] response of the pregnant baboon to cocaine most likely resembles that that occurs in the human pregnancy. Our observations of the pregnant baboon's response to intravenous cocaine lends support to the belief that systemic vasoconstriction is responsible for the adverse perinatal outcome observed in gravid women who abuse cocaine.[1,6-10] The nonhuman primate model used in this study provides an opportunity to improve our understanding of the hemodynamic responses to cocaine ingestion and the determination of cocaine's effect on the cardiovascular system, endocrine responses, and uterine contractility in pregnant nonhuman primates.

We are especially grateful to Willie Clagg for his assistance and wonderful care of the baboons used in this project, to Susan Jenkins for her assistance in data analysis, and to Teresa McCain, Tracey Young, and Karen Moore for their help with this article.

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Discussion

Dr. Robert Resnik, San Diego, California. As of 1985, it was estimated that 30 million Americans had used cocaine as least once, and that 5 million were regular users.[1] Current data would also suggest that usage increase in the general population has been paralleled by that among women in the reproductive age group and those actually pregnant. These alarming and tragic statistics have led to numerous clinical studies over the past several years directed at defining the pregnancy outcomes of pregnant cocaine abusers and their offspring. Such reports show a broad spectrum of unto-

ward perinatal events including premature separation of the placenta, intrauterine growth retardation, preterm labor, fetal distress, neonatal neurobehavioral abnormalities, congenital malformations with a predilection of the genitourinary tract, and isolated reports consistent with fetal cardiovascular accidents.

In 1986, in an attempt to explore the physiologic impact of cocaine in pregnancy, Moore et al.[2] first reported the effects of intravenously administered cocaine on maternal and fetal cardiovascular hemodynamics in the chronically instrumented ewe. They first determined that the dose of cocaine required to produce blood levels in the sheep, similar to those observed in human users, was between 0.3 and 0.5 mg/kg body weight. After intravenous administration of these doses, they noted the rapid onset of sharp increases in maternal mean arterial pressure and a 30% to 40% decrease in uterine blood flow. Within 4 to 5 minutes of cocaine administration there were also significant increases in fetal mean arterial pressure. These findings were confirmed by Woods et al.[3] who also reported increases in uterine vascular resistance, fetal tachycardia, and hypoxemia.

The new findings by Morgan et al. are of obvious importance because they represent the first of their kind in a primate. It is apparent that the physiologic responses observed after cocaine administration in the baboon are quite similar in both magnitude and temporal relationship to those observed in pregnant sheep. Morgan et al. are to be commended for these ambitious studies. Any investigator who has worked with primates appreciates the complexities of maintaining chronic preparations and obtaining reliable data.

When the information that has thus far been obtained from both species is considered, a clearer picture of the pathophysiology of cocaine use in pregnancy begins to emerge. It seems plausible that cocaine produces its deleterious fetal effects by two mechanisms: first, by induction of profound decreases in uterine blood flow with resulting fetal hypoxemia; second, by its rapid placental transfer leading to intense fetal vasoconstriction. These physiologic observations in animal models are consistent with the clinical disorders that have been reported. One might further speculate that fetal vasoconstriction may play a role in the development of congenital defects, although why the genitourinary tract appears to be preferentially vulnerable in human beings remains to be clarified.[4] Recent information derived from a rat model exposed to intraperitoneal cocaine late in the period of organogenesis reveals the acute formation of hemorrhage and edema in the upper extremities, genital tubercle, and upper lip and nose.[5]

I have two questions for Dr. Morgan. The difficulty in obtaining continuous physiologic measurements in the baboon is readily apparent and acknowledged. Nevertheless, I am concerned that the number of observations for each data point never exceeds three or four, and that these data are then expressed as the mean ± SEM. Given this limitation in data points, I would appreciate it if you would comment further on the reliability of the observations.

My second question has to do with the mechanism of cocaine action. As you stated, cocaine has been shown to inhibit the reuptake of norepinephrine at the presynaptic nerve terminal. Moore et al. were unable to inhibit the cocaine-induced increase in mean arterial pressure or decrease in uterine blood flow by pretreatment with the α-blocker phentolamine. More recently, Dolkart[6] reported that α_1-adrenergic receptor blockade with phenoxybenzamine inhibited cocaine-induced maternal vasoconstriction but not the decrease in uterine blood flow. In light of this paradox, I wonder whether you would care to comment on these observations, with particular reference to the effects of cocaine on other neurotransmitters with vasoactive potential.

Again, I want to congratulate you for what surely were difficult and tedious studies. Although small in number, your observations strongly suggest a similarity in the physiologic response to cocaine in the sheep and baboon.

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Dr. Steve N. Caritis, Pittsburgh, Pennsylvania. I do not believe you measured intrauterine pressure, but I wonder whether there were phasic changes in uterine blood flow that might indicate whether there was an increase in intrauterine pressure.

Also, the period of observations seemed to be about 30 minutes. Were any of these animals studied for longer periods of time to determine whether there was a delayed effect on any of the parameters you were measuring? Finally, what was the state of these animals?

Were they agitated or calm? Also, were they horizontal or upright?

Dr. James Woods, Rochester, New York. I would like to focus Dr. Resnik's question even a little bit more specifically. The fact that phentolamine and phenoxybenzamine have been unable to abolish completely the total uterine vascular effect of cocaine raises the possibility that cocaine is working through other neurotransmitters, such as dopamine.

About 2½ years ago your group showed that dopamine does cause uterine artery vasoconstriction and could be a vehicle for this cocaine-induced effect.[1] Have you followed up that study with dopamine blockers to evaluate that issue further?

The second question really relates to the issue of cocaine and pregnancy itself. We know in the sheep that cocaine produces almost a twofold greater cardiovascular response in pregnancy than it does in the nonpregnant state.[2] Moreover, we now know that progesterone seems to be the vehicle that is mediating this unusual drug reaction. In fact, we can reproduce this in the nonpregnant state if one gives progesterone and then gives cocaine.[3]

Have you duplicated any of your infusions either after these pregnant baboons are delivered of infants or more specifically, in the nonpregnant state to compare cocaine-induced cardiovascular effects in pregnant versus nonpregnant baboons?

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Dr. James C. Warren, St. Louis, Missouri. I have one question. I would like some hard data on how your dosages really compare with human users in terms of milligram per kilogram of body weight. I assume they are in the range, but I would like some better information on that, please.

Dr. Morgan (Closing). To answer Dr. Resnik's first question as to the reliability of our observations, I think that any time an investigation is initially performed it needs to be duplicated, either by ourselves with different dosages or by other investigators in a similar animal model. Until that occurs, we really will not know what is truth. We did observe statistical significance with the paired Student *t* test. This statistical test is the most stringent that is used for these particular types of observations.

As to the question, "what is the mechanism of action for the continued uterine blood flow reduction that occurs after blockade of the α_1-adrenergic receptors?" there are two mechanisms that can be related or can be interacting. There are both extrinsic and intrinsic resistances that affect uterine blood flow. What has been specifically investigated with the α_1-adrenergic blockade is the intrinsic resistance, specifically blocking the effects of norepinephrine on the uterine vasculature.

At this meeting in 1987 Dr. Fishburne[1] showed that regional dopamine infusions into the common iliac artery of pregnant baboons caused profound reductions in uterine blood flow. The likely dopamine-mediated increase in intrinsic resistance is also a probable cause for the reduction in uterine blood flow after cocaine administration.

Another possible mechanism is that cocaine could exert a direct effect on the uterine vasculature. Other local anesthetic agents such as lidocaine, administered as a paracervical block, have resulted in profound fetal bradycardia.[2] These observations could be a result of intrinsic vasoconstriction of the uterine vasculature caused by the local anesthetic agents.[3,4]

Increased extrinsic resistance, uterine contractility, could be related to the pharmacologic effects of cocaine. Dr. Griess[5] in 1972 observed that a systemic infusion of norepinephrine caused an increase in uterine activity as measured by intrauterine pressure catheter. This uterine contractile response was somewhat blocked by the administration of phenoxybenzamine in various doses. As to the effect of norepinephrine on the baboon's uterine contractility, the α_1-adrenergic receptor blockade of that effect remains to be determined.

We observed with these cocaine dosages and three others (0.5, 1.0, and 2.0 mg/kg), measuring uterine contractility with intrauterine pressure catheters and electromyographic electrodes, that there is a profound dose-mediated effect on uterine contractility in the nonhuman primate. This effect of cocaine needs to be explored as another possible mechanism for the reduction in uterine blood flow.

As to Dr. Caritis' question on the measurement of intrauterine pressure. The uterine activity did increase and appeared to be related to the dose used. Unfortunately, we did not have the prostaglandin assays finished in time for this particular report.

Observations were obtained over 30 minutes because of study design. However, for the other cocaine doses that I mentioned (0.5, 1.0, and 2.0 mg/kg) we collected data out to 60 minutes and saw similar though greater effects as those observed in this study.

With relation to the state of the animals, each baboon was in her normal upright posture, as in the gang cages. The only difference is that they were wearing a jacket attached to a tether, allowing them freedom to move around in the cage.

The reaction to the cocaine was different from animal to animal. After the higher doses, two of the animals showed twitching of the ears and enlargement of the pupils. However, one observation present in all the baboons was that their buttocks became more intensely red with the increase in the dosage of cocaine. I do not know why this occurs.

To answer Dr. Woods' questions, we are currently preparing to undertake studies that involve dopamine blockade and nonpregnant baboon cocaine infusion studies.

Last, Dr. Warren asked whether the cocaine doses we used in this study are similar to those used by cocaine abusers. We have not obtained these data. The only data that I am aware of are that of Moore et al.[6] who stated that the cocaine blood levels they obtained in their study were similar to peak levels obtained in male volunteers. We have frozen blood samples from which to obtain cocaine levels; however, these assays have not been performed.

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c-*myc* Protooncogene polypeptide expression in endometriosis

Robert S. Schenken, MD, Julia V. Johnson, MD, and Robert M. Riehl, PhD

San Antonio, Texas

The c-*myc* protooncogene and its polypeptide product are important regulators of cell proliferation and differentiation, and ovarian steroids are believed to stimulate growth of various uterine cell types through altered expression of the c-*myc* gene. To determine whether c-*myc* expression may also be involved in the growth and development of endometriosis, we assessed c-*myc* expression in eutopic and ectopic endometrial tissue obtained from women undergoing surgery for endometriosis. Immunocytochemistry using a monoclonal antibody to the c-*myc* protein demonstrated positive staining of glandular and stromal cell nuclei, and cytoplasmic staining of glandular but not stromal cells in both eutopic and ectopic endometrium. These findings suggest that c-*myc* expression may be an important regulator of cell proliferation in endometriotic tissue. (AM J OBSTET GYNECOL 1991;164:1031-7.)

Key words: Endometriosis, c-*myc* protooncogene, c-*myc* polypeptide, immunocytochemistry

Factors regulating the development, growth, and differentiation of endometriosis are poorly understood. Studies to date have suggested that ovarian steroids and estrogen, in particular, serve as the primary mitogenic stimulus for endometriotic tissue.[1] Evidence supporting this includes the clinical observations that endometriosis regresses in hypoestrogenic states,[2, 3] the stimulation of ectopic endometrial growth in animal models treated with exogenous steroid,[3, 4] and the demonstration of both estrogen and progesterone receptors in endometriotic tissue.[5-7] However, these studies also have shown that endometriotic tissue is markedly heterogeneous in its response to ovarian steroids and in its steroid receptor content. For example, ectopic endometrium rarely follows the orderly morphologic changes that occur in eutopic endometrium during the menstrual cycle.[8] In pregnancy, where circulating estrogen and progesterone levels are elevated, most endometriotic implants regress, but some remain unchanged and others may actually increase in size.[9] Although estrogen and progesterone receptors are present in most endometriosis specimens, the concentration of receptor is usually lower than that in eutopic endometrium and several endometriotic implants have no steroid receptors.[5-7] These observations suggest that although ovarian steroids stimulate endometrial cell growth, additional mechanisms, must be involved in controlling endometriotic cellular proliferation.

Recent studies have demonstrated that vertebrate cells contain a number of genes that encode for proteins that are involved in the regulation of cell division and differentiation.[10] These genes are known as protooncogenes because of their similarity to viral oncogenes that are present in the genome of many tumor cells and induce unrestrained cell growth. That the c-*myc* gene, a cellular homologue of the viral v-*myc* oncogene, is a highly conserved gene suggests that it serves a critical function.[11] The gene encodes a nuclear protein that has been positively correlated with cellular proliferation and deoxyribonucleic acid (DNA) synthesis and inversely correlated with cellular differentiation. Although the exact structure and function of the c-*myc*–encoded protein are unknown, it has been postulated that ovarian steroids induce proliferation and differentiation of various cell types via expression of the c-*myc* gene.[12, 13] Accordingly, we questioned whether the c-*myc* gene was also a potential regulator of cell growth in endometriotic tissue and herein report the first immunocytochemical demonstration of the c-*myc*–encoded polypeptide in endometriosis.

Material and methods

Eutopic and ectopic endometrial tissue was obtained from 15 women undergoing laparoscopy or laparotomy for evaluation of infertility or the treatment of pelvic endometriosis. All patients underwent surgery during the proliferative phase of the menstrual cycle. Five patients had ovarian endometriomas and 10 had peritoneal endometriotic implants. Patients ranged from 28 to 40 years of age and none had received recent hormonal therapy.

Histomorphology. Tissue specimens were fixed in 10% formalin and embedded in paraffin. Tissue sections (4 μm) were stained with hematoxylin and eosin for routine histologic examination. All eutopic endometrial specimens were dated according to the criteria of Noyes et al.[14]

From the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio.

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Reprint requests: Robert S. Schenken, MD, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284-7836.

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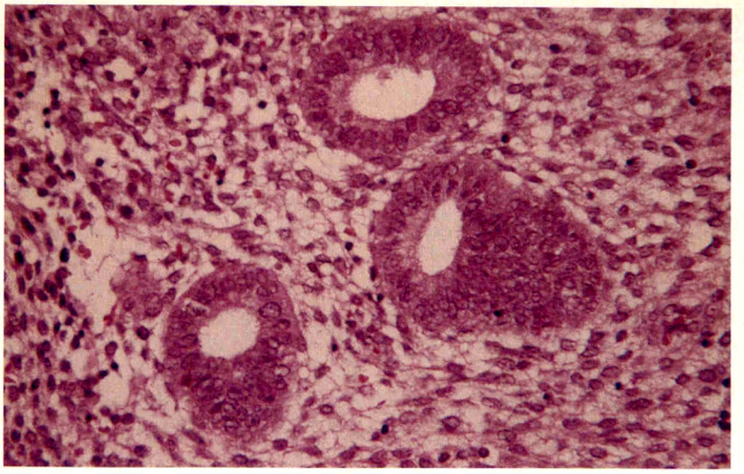


Fig. 1. Proliferative phase eutopic endometrium, stained with hematoxylin and eosin, demonstrating glands and stroma. (Original magnification ×400.)

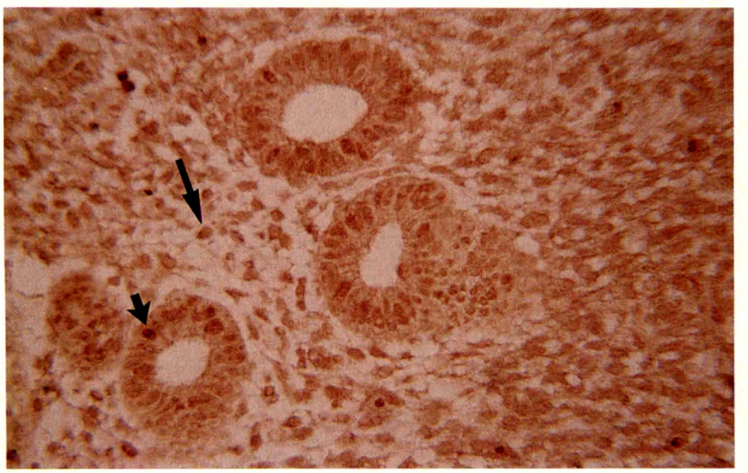


Fig. 2. Immunocytochemical localization of c-*myc* polypeptide in eutopic endometrium section shown in Fig. 1. Formalin-fixed, paraffin-embedded section was incubated with monoclonal antibody to c-*myc* p62 and stained by the peroxidase-antiperoxidase method. Glandular cell nuclei *(small arrow)* demonstrated intense staining with relatively weaker staining of the cytoplasm. In stromal cells only nuclear staining was apparent *(large arrow)*. (Original magnification ×400.)

Immunocytochemistry. Tissue sections were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. Rehydrated sections were pretreated with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity and then 4% goat serum to reduce nonspecific background. The primary antibody (obtained from Oncogene Science, Inc., Manhasset, N.Y.) was produced by immunizing BALB/c mice with the amino acid sequence 408 through 439 of the c-*myc*–encoded 62 kd molecular weight protein (c-*myc* p62) and fusion of SP2 mouse myeloma cells. Sections were incubated with this monoclonal primary antibody at a 1:20 dilution in phosphate-buffered saline solution for 18 hours at room temperature. Immunocytochemical localization was performed using the Vectastain ABC-peroxidase kit (Vector Laboratories, Inc., Burlingame, Calif.) for mouse primary antibody, which uses a biotinylated second antibody, horseradish peroxidase–streptavidin conjugate, and the substrate chromogen diaminobenzidine. Controls consisted of tissue sections incubated with normal mouse serum in the absence of primary antibody. All slides were mounted and examined under bright field.

Results

Histologic dating of eutopic endometrium demonstrated endometrium from the early and mid proliferative phase. Endometrial glands and stroma were present in all endometriotic tissue specimens, but suf-

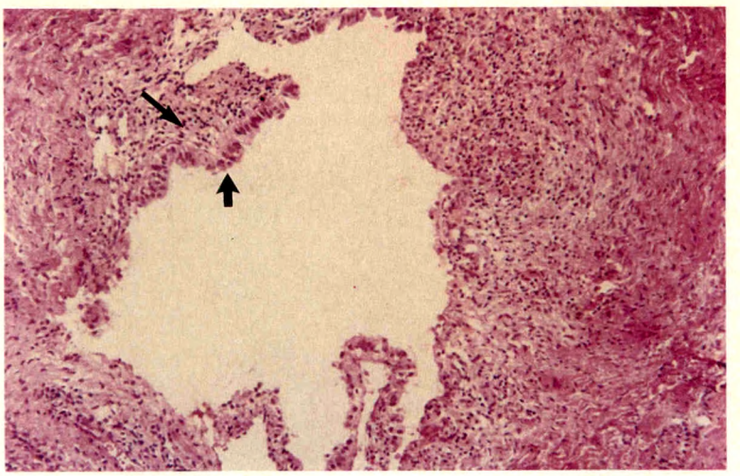


Fig. 3. Peritoneal endometriotic implant section stained with hematoxylin and eosin. Note presence of irregular endometrial gland epithelium *(small arrow)* and adjacent endometrial stromal cells *(large arrow).* (Original magnification ×100.)

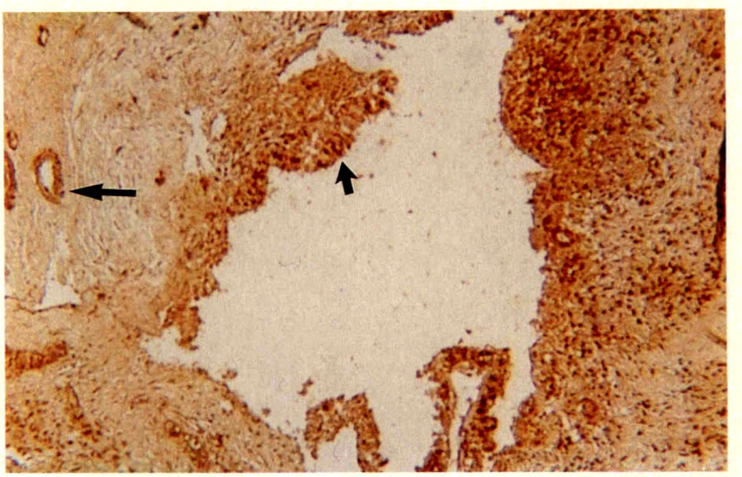


Fig. 4. Immunocytochemical localization of c-*myc* polypeptide in endometriotic implant section shown in Fig. 3. Formalin-fixed, paraffin-embedded section was incubated with monoclonal antibody to c-*myc* p62 and stained by the peroxidase-antiperoxidase method. Note intense staining of nuclei *(small arrow)* in epithelial cells lining lumen of gland. Adjacent endometrial gland nuclei and cytoplasm *(large arrow)* also stained positively. Islands of positively stained stromal nuclei are occasionally present. (Original magnification ×100.)

ficient glandular-stromal architecture to assign dates of the tissue was present in eight specimens. In each case, eutopic and ectopic endometrial specimens were assessed as being early or mid proliferate.

Eutopic endometrial tissue stained positive for c-*myc* p62. Both nuclear and cytoplasmic staining were consistently present in endometrial glands, with nuclear staining being predominant. Nuclei of stromal cells also stained positive, but no cytoplasmic staining was apparent (Figs. 1 and 2). No staining was observed in eutopic endometrium incubated in the absence of primary antibody.

Ectopic endometrial tissue glands in peritoneal implants demonstrated nuclear and occasional cytoplasmic staining (Figs. 3 and 4). Epithelium lining the cyst wall and endometrial glands adjacent to the cyst wall of endometriomas also showed nuclear and occasional cytoplasmic staining (Figs. 5 and 6). In contrast to eutopic endometrial tissue, the intensity of staining in the nuclei and cytoplasm of glandular epithelium in ectopic endometrium was markedly variable. When cytoplasmic staining was present, most sections demonstrated it to be of less intensity than nuclear staining, but some fields showed an equivalent intensity of cytoplasmic and

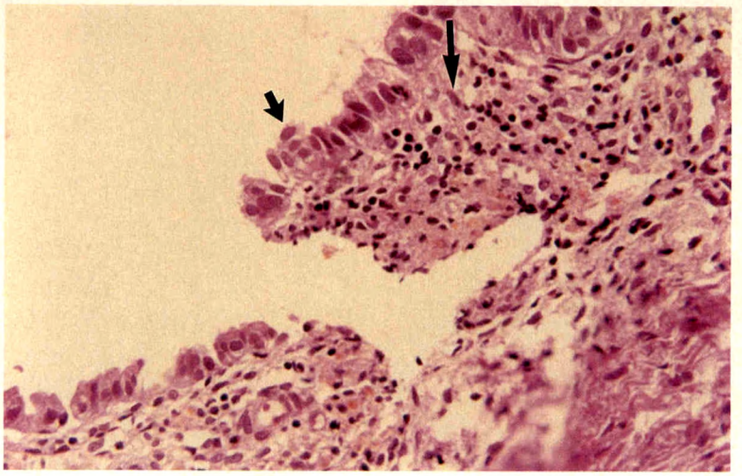


Fig. 5. Section through cyst wall of ovarian endometrioma stained with hematoxylin and eosin. Luminal endometrial cells *(small arrow)* and adjacent endometrial stroma *(large arrow)* are apparent. (Original magnification ×400.)

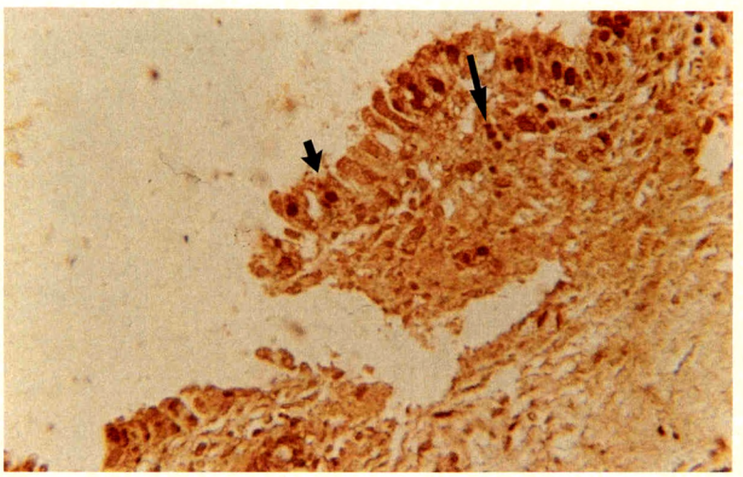


Fig. 6. Immunocytochemical localization of c-*myc* polypeptide in cyst wall shown in Fig. 5. Formalin-fixed, paraffin-embedded section was incubated with monoclonal antibody to c-*myc* p62 and stained by the peroxidase-antiperoxidase method. Note intense staining of nuclei *(small arrow)* in luminal cells and weaker staining of cytoplasm. Stromal nuclei also stained positively *(large arrow)*. (Original magnification ×400.)

nuclear staining. No staining was observed in ectopic endometrial tissue incubated in the absence of primary antibody.

Comment

The aim of this study was to determine whether c-*myc* p62 was present in endometriosis and to obtain preliminary information on qualitative difference in c-*myc* expression in eutopic and ectopic endometrium. Using immunocytochemistry, c-*myc* p62 was identified in the nuclei and cytoplasm of glandular epithelial cells and in the nuclei of stromal cells. Similar findings were observed in eutopic endometrium. When eutopic and ectopic endometrial staining was compared in individual patients, ectopic specimens demonstrated less intense nuclear and cytoplasmic staining and a greater variability in staining among individual glands. We are unaware of any studies that have assessed c-*myc* expression in endometriosis. However, our findings in eutopic endometrium are consistent with one previous report describing changes in endometrial c-*myc* expression during the menstrual cycle.[16] In that study, nuclear staining predominated over cytoplasmic staining in glands obtained during the proliferative phase, but the

converse was observed in secretory endometrium. These findings are consistent with the observation of a nuclear distribution of c-*myc* p62 in actively dividing cells (such as proliferative endometrium) and a cytoplasmic distribution in normal or terminally differentiated cells (such as secretory endometrium).[17] In our study, specimens were obtained only during the proliferative phase. Consequently, it remains to be established whether ectopic endometrium exhibits changes in c-*myc* p62 nuclear-cytoplasmic distribution during the menstrual cycle.

The c-*myc*–encoded protein runs as a doublet with approximate molecular weights of 62 and 64 kd or 64 and 66 kd.[18, 19] The half-life of c-*myc* p62 is very short (20 to 30 minutes), which is consistent for a gene with a role in the regulation of cell proliferation.[19] Although c-*myc* p62 is believed to be primarily a nuclear protein, our immunocytochemistry findings indicate that the protein also is present in the cytoplasm of ectopic endometrial glandular epithelium. Because the catabolism of the c-*myc* protein is unknown, the positive staining of the endometrial epithelial cytoplasm may represent either cytoplasmic synthesis of the protein or a cleavage product of native c-*myc* p62 that was recognized by the monoclonal antibody employed. Western protein blot studies of endometriosis are in progress to investigate the possibility of antibody recognition of its epitope in proteins with lower molecular weights than c-*myc* p62.

Identification of the c-*myc* polypeptide product in endometriosis is an important first step in understanding the role of c-*myc* expression in regulating the development and growth of endometriotic implants. Numerous studies have indicated that the c-*myc* protooncogene and its encoded protein are major components in the regulation of cell proliferation and differentiation.[20] A rapid increase in c-*myc* protooncogene expression is seen after mitogen stimulation of cells, and this c-*myc* induction is associated with transition of cells from a quiescent G_0 state to G_1.[20] Transfection of the c-*myc* gene or microinjection of the c-*myc* protein also will commit quiescent cells to enter the cell cycle.[21, 22] Although the specific function of the c-*myc*–encoded protein is unclear, isolated c-*myc* p62 binds DNA in vitro and may also bind to the nonchromatin structure, termed the nuclear matrix, which is the postulated site of DNA synthesis.[23] Monoclonal antibodies to c-*myc* p62 will reversibly inhibit DNA synthesis and DNA polymerase, further suggesting that the c-*myc*–encoded protein participates in DNA and cell replication.[24] These studies and our demonstration of c-*myc* expression in endometriosis suggest that c-*myc* also is important in regulating transcriptional mechanisms associated with the mitotic cycle of endometriotic cells.

It has been proposed that ovarian steroids mediate eutopic endometrial cell proliferation and differentiation though altered expression of the c-*myc* gene. In oophorectomized mice, estradiol administration rapidly increases c-*myc* messenger ribonucleic acid; this increase is followed by c-*myc* protein accumulation and DNA synthesis in endometrial glandular epithelial cells.[12] Progesterone has similar effects in stromal cells, but blocks estradiol-induced c-*myc* expression in epithelial cells. In vivo treatment with estradiol also induces rapid accumulation of c-*myc* messenger ribonucleic acid in uteri of oophorectomized rats.[13] These studies did not determine whether c-*myc* expression was a direct effect of estradiol or was mediated through hormonally induced paracrine growth factors. The latter possibility is of importance because estradiol increases messenger ribonucleic acid levels of epidermal growth factors and biosynthesis of epidermal growth factor receptors in uterine cell cultures; in some tissues epidermal growth factor induces c-*myc* expression.[12, 25] These findings suggest that endometrial cell growth is regulated by a complex interaction between ovarian steroids and growth factors, with the c-*myc* protooncogene acting as a common mediator of these mitogenic signals.

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Discussion

DR. ROGER D. KEMPERS, Rochester, Minnesota. The c-*myc* protooncogene is expressed in all eukaryotic tissue studied to date, including the growing embryo, the endometrium, and malignant cells. There have been few studies of c-*myc* expression on endometrial samples. Notable have been those by the groups headed by Odom et al. at the Medical College of Georgia (see ref. 16 of article) and Stradtman at the University of Alabama. I am indebted for their counsel in preparing this discussion and also to the reknowned pathologist, Dr. Hazel Gore, for her interpretation of the photomicrographs. Dr. Schenken and his associates have established themselves in this field with the first study of c-*myc* expression in endometriotic tissue. This is a novel study for which they are to be commended.

However, some clarifications and revisions are necessary to make this report more convincing. Permit me to detail several concerns with this study and try to be constructive.

First of all, there is a critical need in the study design for both good positive and good negative tissue controls. The authors studied eight histologic endometriotic sections and an unspecified number of normal endometrial samples. They did not use a negative control other than a negative control for technique by eliminating the antibody. Indeed, it is difficult to obtain negative controls for c-*myc* expression, but germinal centers in the spleen are one of the tissues that do not express this protooncogene. The data from the negative controls for technique cannot be interpreted because the authors did not include their negative control photomicrographs, prepared without antibody. Conclusions regarding positive cytoplasmic staining are not at all conclusive without the control stains for evaluation of background staining. There is a lack of positive control for the histologic or immunohistologic studies other than the use of normal endometrium. Positive control tissue that could be used would be cells from Burkitt's lymphoma, where large quantities of c-*myc* are present or cells from histiocytosis X in the spleen.

The second problem is the lack of evidence of quantitative differences of c-*myc* expression, which was a stated goal. Quantitative analysis of the data by counting the number of grains in the field or performing computerized video imaging was not done. Semiquantitation of the data would strengthen the merits of the study because differential expression in different samples may have important biologic implications.

The third concern relates to several inconsistencies in the interpretation of the photomicrographs of the histologic samples submitted. Fig. 1, which was reported as proliferative endometrium, contains tubular glands, subnuclear vacuoles, and rather edematous stroma; glandular mitoses are not detectable. These findings are not as consistent with early or mid proliferative endometrium as they are with anovulation, a prolonged proliferative phase, or even an early postovulatory state. In Figs. 3 and 4 the large arrows are stated to indicate deep glands, which are not represented in the corresponding hematoxylin and eosin sections. These structures are suggestive of blood vessels and are thus ambiguous. Although the epithelial nuclear staining denoted by the small arrow seems valid, it is curious that such a limited area was selected when more intact glands of epithelium are present in the field. The cells indicated by the large arrows in Figs. 5 and 6 are stated to be stromal cells but could be confused with lymphocytes.

Related to this is a fourth concern. There is a lack of convincing evidence that all of these patients underwent surgery in the proliferative phase of the cycle. The indications for surgery are not given and information about their menstrual histories is limited. Ac-

curate menstrual data should have been obtained at the time of surgery to correlate with the histologic findings. Although the report states that these patients had no recent hormonal therapy, the authors should also provide the minimal time interval since the last such therapy. Since c-*myc* is expressed in mitotically active tissue, positive staining in anovulatory endometrium would not necessarily invalidate the authors' hypothesis. However, the conclusions do pertain to proliferative phase endometrium, and more conclusive evidence should be provided to confirm that normal proliferative endometrium was examined.

Finally, the authors should rewrite two of their conclusions, which are substantiated by the literature but not by their data. Their data show only that c-*myc* protein is expressed in the tissues, appears to take an intranuclear location, and may leak into the cytoplasma. The mere expression of a protein does not support any mediation or regulation. Any direct involvement in regulation of steroid action, by steroids, or as a mediator is only speculation, and this should be clearly stated.

I enjoyed reading this interesting report and hope that the authors will be willing to revise and thus strengthen their manuscript by addressing these concerns about controls, quantitation of the data, photomicrograph interpretation, relevant clinical findings, and appropriateness of conclusions. I look forward to forthcoming contributions in this field by this fine research team.

Dr. Roland A. Pattillo, Milwaukee, Wisconsin. I have one simple question: Would you speculate on the basis for expression of the c-*myc* oncogene protein product in stromal cell in stromal nuclei but not in stromal cell cytoplasm? Is there a significance that is implied from differential expression in the nucleus alone? Is this significance related to the degree of fibrotic reaction to endometrial implants?

Dr. James Schreiber, Chicago, Illinois. You observed a correlation with proliferation, and I wonder whether you have performed this study on luteal phase endometrium just to see if there is a difference in the expression of c-*myc* between what is found in the proliferative phase and in the luteal phase.

Dr. George D. Wilbanks, Chicago, Illinois. I have a similar question, in regard to hyperplasia or tumors of the endometrium.

Dr. Schenken (Closing). I will start with the last question and then address Dr. Kempers' concerns. We have not personally done any studies on c-*myc* expression in endometrial hyperplasia or carcinoma. Other studies have demonstrated that the c-*myc* protooncogene and other cellular oncogenes are expressed in endometrial neoplasia.[1-4]

In the luteal phase overall cellular staining for c-*myc* is somewhat less intense. In the luteal phase epithelial cells, the cytoplasmic staining is somewhat greater than that observed during the proliferative phase. This is probably related to the terminal differentiation of these cells, where the protein is transported from the nucleus into the cytoplasm. In stromal cells only nuclear staining is observed because these cells continue to proliferate throughout the menstrual cycle.

In regard to Dr. Kempers' questions on the indications for surgery, all of these patients were undergoing diagnostic laparoscopy within 10 days of the last menstrual period. All patients were ovulatory as assessed by routine studies: serum progesterone level, endometrial biopsy, menstrual history, etc.

Dr. Kempers questioned the certainty of staining of the glandular components of the ectopic endometrial tissue. In rare cases, we observed staining of blood vessels; these were clearly vessels because red blood cells were present in the luteum. In some cases it is difficult to distinguish between vessels and atrophic glands because the endometrial glandular-stromal architecture is not always as precise as it is in the eutopic endometrium. Even in atrophic or misshapen glands, the absence of endothelial cells and red blood cells in the lumen suggests that these structures are endometrial glands.

We are working on a technique to quantitate c-*myc* expression by computer analysis. This has proved difficult because the endometriotic tissue is heterogeneous and not every microscopic field contains sufficient endometrial glands and stroma.

I appreciate the suggestions regarding the positive and negative controls. In one study the positive control was the staining of eutopic endometrium, which has been demonstrated previously. The negative control was the lack of staining of sections incubated in the absence of primary antibody. It is true that the c-*myc* protooncogene is expressed in almost all tissues to date; we were interested in whether c-*myc* protooncogene was present in this estrogen-sensitive tissue and its potential role in cell proliferation.

It is currently unclear whether the c-*myc* protooncogene is a critical regulator of cell proliferation in endometriosis. We are developing studies to look at this in explanted cultures of human endometrium and in a rat model. On the basis of previous studies of eutopic endometrium in rats, we believe that c-*myc* expression will be demonstrated to be an important regulator of endometriotic tissue growth.

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Serum interleukin-6 levels correlate with disease status in patients with epithelial ovarian cancer

Jonathan S. Berek, MD, Catherine Chung, BS, Klara Kaldi, BS, Joanna M. Watson, BSc, Reba M. Knox, MS, and Otoniel Martínez-Maza, PhD

Los Angeles, California

Interleukin-6 is a pleiotropic cytokine with a wide range of effects, including induction of B-cell and cytotoxic T-cell differentiation, and induction of acute phase reactant production by hepatocytes. Interleukin-6 also can act as an autocrine growth factor in malignancy. Various cell types produce interleukin-6, including T and B cells, monocytes, fibroblasts, and some solid tumor cells. In previous work we detected the production of substantial amounts of interleukin-6 by human ovarian cancer cells, including the ovarian cancer cell lines CAOV-3, OVCAR-3, and SKOV-3, and several primary ovarian tumor cultures. In this study we retrospectively examined 90 separate serum specimens for interleukin-6 in 36 patients with epithelial ovarian cancer. The mean serum interleukin-6 concentration of those ovarian cancer patients with macroscopic disease ($n = 57$) was 0.26 ± 0.04 U/ml (mean ± SEM). Healthy adult donors have interleukin-6 serum levels of 0.12 ± 0.03 U/ml. Sixteen of 21 ovarian cancer patients with macroscopic disease (76%) had elevated (>0.20 U/ml) levels of serum interleukin-6, with levels approaching 1 U/ml in some patients ($p < 0.01$). Of those nine patients with bulky tumor (residual >2 cm), eight (89%) had an elevated interleukin-6 level (mean, 0.31 ± 0.05), while eight of 12 (66%) with minimal residual disease (<2 cm) had elevated levels. Only two of 15 (13%) patients who were in clinical remission and who had microscopic disease had elevated values. Of the 36 patients, 22 were CA 125 negative (<35 U/ml), and of these, four had elevated interleukin-6 levels. Of the 14 patients with an elevated CA 125 level, 12 (86%) had elevated interleukin-6 levels. In those 16 patients in whom serial levels of interleukin-6 were measured, rising levels were found over a 3 to 4 month interval in nine (56%); this correlated with tumor progression. Furthermore, the subsequent survival of patients was shown to correlate with the level of interleukin-6, such that patients whose levels were elevated >0.20 U/ml interleukin-6 survived a mean of 12.5 months, compared with 27.2 months for patients with normal levels ($p < 0.001$). These data support the concept that interleukin-6 may be a useful tumor marker in some patients with epithelial ovarian cancer, as it correlates with the tumor burden, clinical disease status, and survival. (Am J Obstet Gynecol 1991;164:1038-43.)

Key words: Human ovarian cancer, cytokines, interleukin-6, serum levels

Of all gynecologic malignancies, ovarian cancer has the lowest overall survival rate. Most patients have advanced stage disease at the time of diagnosis.[1] Monitoring and detection of subclinical disease is limited because the majority of diagnosed patients have no measurable disease while they are undergoing treatment. Therefore suitable biomarkers are needed. The ovarian cancer–associated marker, CA 125, has been the most well characterized, and it has been useful in helping to monitor the course of disease in many patients with nonmucinous epithelial malignancies.[2] Also, ovarian malignancies frequently metastasize throughout the peritoneal cavity before the development of symptoms,[1] and thus better methods of early detection are needed.

Interleukin-6 (IL-6), also known as BSF-2, interferon beta-2, hybridoma/plasmacytoma growth factor, and 26 kd protein,[3-6] is a pleiotropic cytokine involved in numerous, diverse biologic functions.[7-9] IL-6 has been shown to be produced by human tumor cells including cardiac myxoma cells,[10] myeloma cells,[11] and acquired immunodeficiency syndrome–associated Kaposi's sarcoma cells.[12] IL-6 can augment the growth of myeloma cells or Kaposi's sarcoma cells in an autocrine loop.[11, 12] Recent evidence has shown that IL-6 is produced by both human epidermal cells and epidermoid carcinoma

From the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, and the Department of Microbiology and Immunology, University of California, Los Angeles School of Medicine and the Jonsson Comprehensive Cancer Center.

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Reprint requests: Jonathan S. Berek, MD, Department of Obstetrics and Gynecology, University of California, Los Angeles, Los Angeles, CA 90024.

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cell lines[13] as well as other epithelial tumors including bladder carcinoma cells[14] and renal cell carcinoma.[15] Because the majority of ovarian carcinomas arise from the coelomic epithelium that covers the ovarian surface,[1] we examined the production of IL-6 from several established ovarian carcinoma cell lines and from freshly excised epithelial ovarian tumors.[16] We showed that three of four established ovarian carcinoma cell lines, as well as primary cultures taken from ovarian tumors, secrete IL-6. By all criteria examined, this IL-6 was indistinguishable from that produced by other epithelial, lymphoid, or monocytoid cell lines. Furthermore, these epithelial ovarian tumor cell lines may produce IL-6 as an autocrine growth factor.

Recent data have shown that ovarian stroma and follicles are rich in IL-6 gene expression; this appears to be related to angiogenesis.[17] Thus IL-6 may function as a paracrine growth factor in the normal ovary. In tumorigenesis, the proliferation of new blood vessels is a critical step,[18] and therefore the overexpression of IL-6 might serve as an internal promoter of neoplasia in a manner that helps to support the growth of the tumor. With the proliferative activity, IL-6 might be secreted in large quantities into the peritoneal cavity and then into the systemic circulation.

Tumor development has been associated with either oncogene expression and amplification or inappropriate growth factor regulation.[19-21] There has been little success in correlating significant oncogene expression and amplification with ovarian cancer,[20] with the exception of the recently identified HER-2/*neu* oncogene,[19] which encodes an epidermal growth factor–like receptor,[21] has been found to be overexpressed in approximately 30% of ovarian malignancies, and correlates with a poor clinical prognosis. These findings support the concept that the development and progression of ovarian cancer may be related to inappropriate growth factor regulation. IL-6 is a growth factor that offers potential relevance to the understanding of ovarian malignancies.

On the basis of this rationale, we sought to characterize the production of this cytokine by ovarian tumors and to provide information on the role of IL-6 as a growth factor for ovarian cancer. The preliminary finding of elevated levels of circulating IL-6 in the serum of patients with epithelial ovarian cancer stimulated our efforts to correlate the patients' tumor status with the levels. These data might provide a new serum marker that can be used for the diagnosis and treatment of ovarian carcinoma.

Material and methods

Patients. Patients with histologically documented epithelial ovarian cancer were studied. Blood was drawn at the time of either hospital admission for surgery or therapy, or at outpatient visits. In all cases the physical examination was performed by a physician and the status recorded in the chart. Serial evaluations permitted the determination of whether the patient was in clinical remission or whether measurable disease was progressing, regressing, or stable. When available, radiographic evidence of disease by computed tomographic scan was monitored by repeat scans. The extent of residual disease was measured by laparotomy and was quantitated by the surgeon. Survival of patients was measured in months from the time of surgery to latest time of follow-up or death.

Serum samples. Aliquots of blood were obtained by venipuncture in 15 ml siliconized glass tubes without coagulants. The blood was allowed to clot and centrifuged at 1000 rpm for 10 minutes; then the separated serum was removed. Serum specimens were assayed immediately or stored at −70° C, thawed, and assayed. We have found that a single freeze-thaw does not affect the levels of measurable IL-6. The specimens were handled consistently and processed rapidly to avoid spurious production of IL-6 by the patients' leukocytes.

IL-6 assay. Serum specimens were determined to have IL-6 biologic activity by bioassay using the IL-6 dependent murine hybridoma cell line MH60.BSF-2 (kindly provided by Dr. T. Kishimoto and Dr. T. Hirano, Osaka, Japan) as previously described.[16, 22] Briefly, MH60.BSF2 cells are maintained in RPMI-1640 medium supplemented with 10% fetal calf serum, 1% antibiotics, 1% glutamine, 2% 2-mercaptoethanol, and 5 U/ml recombinant human IL-6 (rhIL-6, provided by Dr. T. Kishimoto). Duplicate serum samples were serially diluted in 96-well flat bottom plates (Nunc, Inc., Roskilde, Denmark). A total of 1×10^4 washed MH60.BSF2 cells was added per well, and the plates were incubated at 37° C for 42 hours. The cells were pulsed with 1 μCi ^{3}H-thymidine (ICN Biochemicals, Irvine, Calif.) for 6 hours and harvested onto glass membrane filters; then the radioactivity was counted.[15] IL-6 levels were determined by comparison with MH60.BSF2 cell proliferation induced by samples that have been exposed to known quantities of rhIL-6. We have shown that the addition of anti-IL-6 antibody eliminates IL-6–induced IL-6/MH60.BSF-2 cell proliferation.[16] Serum specimens were heat treated (56° C, 30 minutes) to remove nonspecific IL-6 inhibitory activity.[23] Interassay variation was assessed, and the mean variation was 20%. The assay was performed by laboratory personnel unfamiliar with the clinical status of the patients.

CA 125 assay. CA 125 was measured in the serum using the standard radioimmunometric assay with a murine monoclonal antibody (OC 125) and measured in a γ-scintillation counter using duplicate specimens.[24]

Statistics. Statistical analysis was done by using Student's *t* test.

Table I. IL-6 serum levels by bioassay in patients with epithelial ovarian cancer

Disease status	*Patients (No.)*	*Specimens (No.)*	*IL-6 levels Elevated**	*Mean ± SEM*
Macroscopic	21	57	16 (76%)	0.26 ± 0.04†
Bulky (>2 cm)	9	27	8 (89%)	0.31 ± 0.05‡
Minimal residual disease (<2 cm)	12	30	8 (67%)	0.23 ± 0.04†
Microscopic	15	33	2 (13%)	0.09 ± 0.03
TOTAL	36	90	18 (50%)	0.24 ± 0.04†
Control	12	12	2	0.12 ± 0.03

*Sensitivity (macroscopic), 0.76; microscopic plus macroscopic, 0.50.

†$p < 0.05$, versus control.

‡$p < 0.01$, versus control.

Table II. Correlation of serum IL-6 levels and CA-125 levels in patients with epithelial ovarian cancer

CA 125 level	*No. of patients*	*Elevated IL-6 level*
Normal (<35 U/ml)	22	4 (18%)
Elevated (>35 U/ml)	14	12 (86%)

Correlation coefficient = 0.84.

Results

The IL-6 levels of bioassay from 36 patients with epithelial ovarian cancer are presented in Table I. IL-6 levels in 90 separate serum specimens were measured. The mean values of the initial levels are correlated with the extent of disease at the time of exploratory laparotomy. The mean serum level of those ovarian cancer patients with macroscopic disease ($n = 57$) was 0.26 U/ml. Sixteen of 21 patients (76%) in this group had elevated levels of serum IL-6 (>0.20 U/ml), with serum levels approaching 1 U/ml in some patients. On the basis of the IL-6 value of 0.12 ± 0.03 U/ml in the serum of healthy adult female volunteer donors tested ($n = 12$), the values in cancer patients are elevated significantly.

Of the nine patients with bulky tumor (residual >2 cm maximum tumor dimension), eight (89%) had an elevated IL-6 level (mean, 0.31) ($p < 0.01$), whereas eight of 12 (66%) with minimal residual disease (<2 cm maximum tumor dimension) had elevated levels ($p < 0.05$). Only two of 15 (13%) patients who were in clinical remission and who had microscopic disease had elevated serum values.

CA 125 levels were correlated with IL-6 levels. Of the 36 patients, 22 were CA 125–negative (<35 U/ml), and of these, four had elevated IL-6 levels. Of the 14 patients with elevated CA 125 levels, 12 (86%) had elevated interleukin-6 levels (Table II). Fifteen of the 36 patients tested had microscopic disease only at the time of measurement, and all but one of these patients had a negative CA 125 level.

Serial IL-6 levels were measured in 16 patients. Rising serum levels were found over a 3 to 4 month interval in nine patients (56%), and in all cases this correlated with tumor prognosis. Of the other seven serial levels, five remained within the same range and two decreased; these patients had subclinical disease and remained in clinical remission during this interval.

Patient survival was correlated with levels of IL-6 at the time of laparotomy. Subsequent survival of patients was shown to correlate with the initial level of IL-6, so that patients whose levels were elevated >0.20 U/ml survived a mean of 12.5 months, compared with 27.2 months for those patients with normal levels ($p < 0.001$).

Comment

Our study shows that the serum of many patients with epithelial ovarian cancer contains levels of the growth and differentiation factor IL-6 that are significantly higher than those found in normal controls. The levels correlate with the extent of residual disease, such that those patients who have a large tumor burden also tend to have high IL-6 serum levels. Furthermore, in patients who develop evidence of clinical progression, the IL-6 levels become elevated. The survival of patients from the time of surgery correlates with the level of elevation, suggesting that overexpression of this growth factor might be prognostic in epithelial ovarian cancer. Thus these data suggest that serum IL-6 measurements may be a useful biomarker for epithelial ovarian cancer.

Ovarian malignancies constitutively produce IL-6. We have reported previously that most epithelial ovarian cancers studied in vitro produce significant levels (>10 ng/ml) in the supernatants of primary ovarian tumor cultures.[16] Tabibzadeh et al.[25] examined IL-6 immunoreactivity in human tumors by immunohistologic staining and found that the majority of human tumors of epithelial or mesenchymal origin stain positive. Furthermore, three well-characterized human epithelial ovarian carcinoma cell lines, CAOV-3, NIH-OVCAR-3, and SKOV-3, constitutively produce biologically active and antigenically related IL-6.[16] The IL-6 produced by these ovarian cancer cell lines has a molecular weight of approximately 24 kd, and exhibits some of the molecular weight heterogeneity often associated with IL-6 produced by nonovarian epithelial cells.[13, 16] All of the primary ovarian tumors and ovarian cell lines that produce IL-6 activity are epithelial adenocarcinomas,

and the only ovarian carcinoma cell line tested that does not produce any IL-6, PA-1, was originally derived from a teratocarcinoma,[26] a germ cell tumor.

The secretion of IL-6 into the blood of patients with epithelial ovarian cancer is presumably based on the up-regulated expression of the IL-6 gene within the ovarian epithelial and stromal cells of these malignancies. A recent report by Motro et al.[17] suggests that IL-6 is a paracrine growth factor secreted by the epithelial and stromal cells of normal ovaries and by the ovarian follicles and that IL-6 gene expression is associated with angiogenesis within the stroma. On the basis of these data, we postulate that, during the process of tumorigenesis, IL-6 gene expression helps to promote tumor growth and excess IL-6 is secreted into the peritoneal cavity and blood of the patient.

Although IL-6 has been implicated as an autocrine growth factor in other tumor systems,[11, 12, 15] we have reported that added exogenous IL-6 does not lead uniformly to the increased proliferation of ovarian cancer cells and that anti–IL-6 serum does not inhibit ovarian tumor cell growth.[16] The lack of proliferative response to exogenous IL-6 suggests that these ovarian carcinoma cell lines are not producing this cytokine as an extracellular autocrine growth factor.[16] However, the possible role of ovarian cell–produced IL-6 as an intracellular autocrine growth factor for ovarian cancer cells or as an exocrine or paracrine growth factor for other cells of the peritoneal cavity is interesting, because of the high levels of the cytokine that are produced and retained within the peritoneum and the lack of metastasis outside of the cavity. It has been proposed by Tamm et al.[27] that IL-6 may promote tumor metastasis and invasiveness because exogenous IL-6 has been shown to increase motility and decrease adherens junctions in the breast carcinoma cell lines T47D and Zr-75-1. It is not known whether autonomously produced IL-6 would affect the motility and adherence of the ovarian cancer cell lines, but this phenomenon could explain the extensive peritoneal carcinomatosis seen in patients with ovarian carcinoma. Also, IL-6 could play an important role in the establishment of autonomous tumor by acting as an angiogenesis-inducing factor.[17]

We have previously reported that there are extremely high levels of this cytokine within the peritoneal cavity ascitic fluid of patients with ovarian cancer.[16] The high concentrations of IL-6 within a confined and localized region reported here are not unique, as high levels (>1000 U) have been reported to be present in amniotic fluid,[28] in the synovial fluid of rheumatoid arthritis patients,[29] and in localized regions of acute bacterial infections.[30] Our preliminary studies suggest that these high levels do not contribute directly to the immunosuppressive effects of ascitic fluid.[31] It is not clear what immune functions IL-6 plays in such localized regions and whether high, sustained concentrations actively suppress or enhance other immune functions or whether they induce a state of nonresponsiveness through an indirect means.

The presence of high levels of IL-6 in several patients who have a low level of the surface antigen CA 125 suggests that the assay might contribute to the monitoring of such patients. However, because the correlation coefficient between the two markers is high, the lack of complementarity might diminish the utility of IL-6.

The relevance of tumor differentiation to IL-6 production cannot be drawn from this study, as there are too few with each tumor type, but it would be interesting to examine this to determine if such a correlation exists. The ovarian carcinoma marker CA 125 has been demonstrated to be associated with approximately 85% of serous ovarian tumors, 70% of undifferentiated tumors, and 30 to 40% of mucinous tumors.[2]

Evidence of Boltz et al.[20] regarding the lack of any significant amplification of the c-*ras*-Ki oncogene in ovarian tumors suggests that ovarian tumor progression may be related to abnormal growth factor regulation as opposed to inappropriate oncogene expression and amplification. However, evidence relating abnormal growth factor regulation to ovarian tumor progression has been demonstrated by the amplification and overexpression[19] of an epidermal growth factor–like receptor[21] encoded by the HER-2/*neu* oncogene. Also, recent reports have shown that macrophage colony–stimulating factor, the receptor for which is a product of the c-*fms* gene locus, also is secreted by many ovarian epithelial tumors.[32] While the unregulated expression of IL-6 has been implicated in the pathogenesis of numerous diseases, including some lymphoid malignancies,[33] it remains to be determined what role such deregulated expression of IL-6 has in the development and progression of ovarian cancer. However, the unregulated expression of this growth factor in the development of ovarian cancer may offer a means of abrogating tumor proliferation by regulating the factor or its genetic locus.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. John Boyce, Brooklyn, New York. The search for efficient ovarian tumor–associated markers has been tedious and difficult. This study, using a cohort of 36 patients with 90 specimens, attempts to show that IL-6 correlates well with tumor burden, clinical disease status, and survival and consequently should be considered seriously as a useful tumor marker.

The role of a tumor associated–marker may be measured in two areas, the screening for the malignancy and the monitoring of the results of therapy. The information presented today shows a statistically significant difference in the IL-6 levels between the patients with ovarian carcinoma and the controls. There is an excellent correlation with gross disease and IL-6 in 21 patients. There is, however, a disturbing lack of a difference between 15 patients with microscopic disease

and 15 controls. No data were offered on the specificity or sensitivity of IL-6 measurements in the patients with ovarian cancer. Since the production of IL-6 is increased in bacterial infections and rheumatoid arthritis, an analysis of the specificity and sensitivity is essential to a conclusion of the efficacy of IL-6 as a marker for screening purposes.

The evaluations of IL-6 as a marker for monitoring the results of therapy is less complicated since the interindividual considerations are eliminated. Serial measurements were performed in 16 patients, and there was an excellent correlation with the clinical behavior of the patients. Thus IL-6 may be used very accurately for monitoring the progress of therapy. The poor correlation with microscopic disease will again limit severely the use of IL-6 in determining whether there is a complete response. It is interesting that the same limitation applies to CA 125, i.e., the greatest opportunity for use is in those patients with subclinical disease. IL-6 does not appear to have an advantage over CA 125, and there is also no complementary effect of IL-6 to CA 125.

The study design is simple and solid. The statistical analysis is adequate for the study. I wish to compliment the authors for the innovative work that they have accomplished in this study, taking the initiative in exploring new markers in ovarian carcinoma, a disease where 60% of the diagnoses are made in stages III and IV with 5-year survival of 20% and 10%. I have two questions for the authors: (1) How many of the 36 patients with ovarian cancer were untreated on entry to the study? (2) What were the epidemiologic characteristics of the control group?

Dr. Howard W. Jones III, Nashville, Tennessee. I would like to ask if this has the same problem as CA 125, that is, a positive test in other clinical situations, such as endometriosis or pelvic inflammatory disease, which would further limit its value as a screening test?

Dr. J. Donald Woodruff, Baltimore, Maryland. I think we certainly need steps like this because we're making very little progress in the early diagnosis of ovarian cancer.

When you state that this test was positive for ovarian cancer, are you referring to mucinous tumors and epithelial tumors of all types? Was there any difference in the positivity between the various histologic types?

Dr. Berek (Closing). I would like to thank Dr. Boyce for his thoughtful comments.

There were 19 patients in the group of 36 whose serum IL-6 levels were measured prior to the first chemotherapy. Most of the 16 patients who were monitored serially came from this group. Initially these patients were in a study where we measured serial CA 125 levels, so we reexamined those frozen sera. Of the patients measured serially, 15 had not received prior therapy before the first measurement. Many of the others had microscopic disease and were on intraperitoneal chemotherapy protocols when the serum IL-6 levels were measured. Therefore they had a laparotomy before the evaluation and a laparotomy at the end so that we could correlate the data.

The control group is a matched group of women without any evidence of disease. To determine the specificity and to address the question that Dr. Howard Jones raises about whether this is positive in other conditions, we would have to study a cohort of women with a variety of different benign conditions. We have not done this yet.

Regarding the sensitivity of this study, the value is 0.5 for the entire group, although this is 0.76 for those with macroscopic disease. This corresponds to sensitivities of 0.6 and 0.87, respectively, for core CA 125 measurements on the same sera.

Currently we are examining ways to improve the sensitivity and specificity of the assay. We are developing a refined IL-6 assay using a more specific enzyme-linked immunosorbent assay. Also, we plan to measure IL-6 levels in many fresh serum specimens and a larger control group.

To answer Dr. Woodruff's question, we did not find a particular correlation with pathologic results, perhaps because we have too few in the sample. There were several patients with mucinous tumors who did have positive IL-6. A known drawback of CA 125 is that many of those patients have negative assays.

However, it may well be that IL-6 is more important in the understanding of the fundamental tumor growth rather than as a useful tumor marker. Currently we are evaluating the elaboration and control of IL-6 expression by using antisense oliogonucleotides to modify gene expression of the growth factor. Also, we are using a murine model to study therapeutic approaches with monoclonal antibodies that are directed either toward the IL-6 gene product and other growth factors or their receptors. Since IL-6 is a constitutive product of the ovary and presumably related to angiogenesis, we hope that abrogation of expression of the genetic locus of the growth factor might delimit tumor proliferation or growth.

Ultrasonography in obstetrics and gynecology: The state of the art in 1990

Ultrasound panel

Introduction

Of the many technologic developments of the past quarter century, none has become as thoroughly integrated into the practice of obstetrics and gynecology as diagnostic ultrasonography. It is based on technology developed for military purposes in World War II, but its potential applicability to the solving of the mysteries of the uterus and its contents was first recognized by a canny Scot, Ian Donald, who played the role of St. Paul in disseminating and popularizing a new religion. Other "apostles" rose up and joined the movement, until today the pervasive influence of ultrasonography on obstetrics and gynecology has altered the discipline in fundamental and irrevocable ways. Practitioners under the age of 40 or 45 have difficulty imagining that pregnancy can be managed or that gynecologic disease can be dealt with unless high-frequency sound waves have been made to traverse a woman's body.

Ideally, development of technology should include the consideration of several important questions, with reasonably firm answers required before its incorporation into clinical use. As the first priority, is it safe? Then, what are its uses and limitations? Finally, what is the cost and is it cost-effective? In the case of ultrasonography, as indeed with other technologic developments in medicine, this sequence was not followed. Instead, the technique was introduced and adopted widely, no doubt influenced by the commercial interests of the makers and users of the equipment, before there was much in the way of logical consideration of these essential questions. Happily, in the case of ultrasonography, evidence accumulated over the past 20 years should be sufficient to convince all but the most skeptical of its safety. Much is now known about its use and limitations, and a consensus seems to be developing as to the circumstances where it is and is not helpful, although the issue of using routine ultrasonography for all pregnant women remains a thorny one. Many individuals have argued for its incorporation into routine antepartum care, although the official position (i.e., the 1984 National Institutes of Health Consensus Development Conference) calls for usage only "on indication." Much of this controversy hinges on the last question, cost effectiveness, which has not really been addressed in any serious way.

The American Gynecological and Obstetrical Society decided last year to ask a panel of its Fellows to address a specific clinical issue facing the specialty with a "position paper" to place that issue into current perspective and to identify future needs. Ultrasonography was an obvious choice for the first of these symposia. The members of the panel, Roy M. Pitkin, James A. Low, and J. Robert Willson, were selected presumably as interested observers, who together cover the breadth of the discipline but who lack any vested interest in ultrasonography.

The time allotted on the program meant that only a limited area of the field could be addressed. Through correspondence, telephone calls, and one meeting, the panel attempted to identify the most important aspects for consideration. A list of 12 or 15 potential topics was developed, discussed, and voted on. By this means, the panel arrived at three topics to present to you today, what we believe are the important issues regarding the use of ultrasonography in obstetrics and gynecology: (1) screening and detection of congenital malformation, (2) Doppler velocimetry, and (3) ultrasonography in gynecologic diagnosis.

Screening and detection of congenital malformation

Roy M. Pitkin, MD
Los Angeles, California

Congenital malformations often occur sporadically and unpredictably, and a method of population screening to identify at-risk pregnancies would be of great value. Routine ultrasonography has some appeal in this regard. Unfortunately, the literature indicates a relatively poor sensitivity, with usually 30% or less of malformations identified or suspected by this means. Substantially better results have been described with ultrasonography targeted on the basis of screening ultrasonography or clinical findings, but these studies involve highly selected subjects examined carefully by experienced ultrasonographers, and their results are not relevant to population screening. A questionnaire survey of experts in the field indicated disagreement with the separation of screening and targeted ultrasonography, widely varying estimates of the accuracy of either, and average charges of $209 and $263, respectively. Theoretical considerations of the various types of malformations, their frequencies, and the likelihood of ultrasonographic detection reveal a minority of defects in which ultrasonography is both accurate and useful clinically. Ultrasonography seems to be of little potential as a means of screening for congenital malformation because of relatively low sensitivity and uncertain cost effectiveness. (Am J Obstet Gynecol 1991;164:1045-8.)

Key words: Ultrasonography, congenital malformation

The prevalence of major congenital malformation (i.e., defects either incompatible with life or severe enough to interfere with normal living) is about 2% to 3%. For practical purposes the only "treatment" available is abortion; therefore methods of screening and detection generally must lead to a diagnosis before the midpoint of gestation. For this reason most screening programs involve testing at about 16 weeks' gestation.

Although abortion is the most obvious response to the diagnosis of a severely malformed fetus, it is not the only one. For women who choose to carry the pregnancy to term, knowledge of the fetal condition can influence obstetric and neonatal care, such as where and how delivery is to be accomplished. This type of information also can allow time for emotional adjustment and avoid the acute psychologic trauma accompanying the birth of an unexpectedly malformed infant. Finally, although the capacity to treat the fetus directly and thereby ameliorate or obviate the effects of a congenital abnormality is minimal at present, there is reason to believe this may become more applicable in the future.

A number of factors influence the usefulness of ultrasonography in the screening and detection of congenital malformation. Chief among these is the stage of gestation at which ultrasonography is performed. As outlined previously, the general rule is "the earlier the better," with respect to how the information will be used. However, the study's accuracy will generally increase as gestation advances, so from this perspective the rule is "the later the better." Determining factors of equal or even greater importance are the skill and experience of the ultrasonographer and the length of time spent in examination.

Literature review. Most of the arguments in favor of routine ultrasonography in pregnancy involve identifying gestational length accurately and recognizing multiple pregnancy. However, the potential also exists for the detection of congenital malformation. Thacker[1] reviewed the randomized clinical trials of routine ultrasonography reported before 1985; several more have been reported subsequently.[2,3] These studies have generally not found any improvement in pregnancy outcome, suggesting that either malformations have not been identified in sufficient quantities to influence outcome statistics or they have been identified but not aborted.

Several reports have addressed anomaly detection specifically. All involved retrospective analysis. Persoon and Kullander[4] described an experience with 43,000 scans in 22,400 patients; only 24% of anomalous fetuses were identified early enough to permit termination of pregnancy. Lys et al.[5] analyzed 8316 low-risk pregnan-

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Reprint requests: Roy M. Pitkin, MD, Department of Obstetrics and Gynecology, University of California, Los Angeles, 10833 Le Conte Ave., Los Angeles, CA 90024-1740.
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Table I. Questionnaire survey of screening and targeted ultrasonography

	Screening	*Targeted*
Malformations suspected (%)		
Mean	66	87
SD	14	12
Range	37-90	50-99
Total charge ($)		
Mean	209	263
SD	67	94
Range	66-335	110-663

cies scanned routinely, with only 14% of malformations identified; the disappointing results were thought to reflect the fact that the examinations were not done by highly trained personnel and were not "targeted" to anomaly detection. In a small but carefully analyzed series of routine ultrasonographic scans done for no apparent clinical reason, Li et al.[6] calculated a sensitivity of 38% and a specificity of 99% for screening a low-risk population for fetal malformation. Similar results came from a study by Levi et al.[7]: sensitivity, 34.4% for all defects and 54.9% for major and detectable ones; specificity, 99.7% in both cases. Thus these reports are fairly consistent in indicating that ultrasonography for screening has a rather poor sensitivity but an excellent specificity. In other words, it is not good in detecting the malformed fetus but very good in ruling it out.

Compared with screening examinations, ultrasonography targeted specifically to possible fetal malformations has been reported to be substantially more accurate. Such studies typically are prompted by findings on screening ultrasonography, clinical characteristics, or history indicating high-risk status. Sabbagha et al.[8] reported on 615 women who underwent targeted imaging, usually but not always before 24 weeks' gestation; sensitivity was 96% and specificity 99%. Manchester et al.[9] described the experience of the University of Colorado program, in which 257 consecutive patients were referred at 12 to 40 weeks' gestation because fetal malformation was suspected on ultrasonography elsewhere. The program's evaluation included fetal echocardiography, if indicated, and involved a multidisciplinary team. Sensitivity was 99% and specificity 91% when each fetus was considered as a unit; however, 37% of the anomalous infants had malformations not suspected on ultrasonographic examination, in addition to defects which had been identified ultrasonographically. Hobbins et al.[10] and Hill et al.[11] reported experiences that, while not presented in sufficient detail to calculate statistics, seem generally similar. It must be emphasized that all these seemingly excellent results came from individuals with vast experience in ultrasonography who presumably devoted substantial amounts of time and attention in a thorough evaluation of each case. Moreover, the study populations must have been highly selective. Somewhat in contrast with this optimistic picture is a collection[12] of six anecdotal cases in which misinterpretations and errors in diagnoses led to unfortunate outcomes. Most often the mistake involved early delivery, which the authors pointed out is seldom necessary for the anomalous fetus.

Questionnaire survey. To sample current opinion of ultrasonographers about screening and detection of fetal malformation, a simple questionnaire was sent to 46 individuals regarded as experts in obstetric ultrasonography by virtue of articles they had written. Most, but not all, of those surveyed are in full-time academic practice. The questionnaire was divided into two parts, one dealing with screening, or level I, ultrasonography and the other with targeted, or level II, ultrasonography. Each respondent was asked to estimate what percentage of "clinically significant fetal malformations" would be suspected by screening ultrasonography and diagnosed by targeted ultrasonography, with gestational age unstated. He or she also was asked about the total charge for these procedures in his or her unit.

Questionnaires were returned by 11 of 20 radiologists and 25 of 26 obstetrician-gynecologists, for an overall return rate of 78%. None of the differences between the two medical specialties was statistically significant, and the results are presented together. A number of respondents regarded the distinction between screening, or level I, and targeted, or level II, ultrasonography as artificial and inappropriate, presumably reflecting the attitude of national organizations.[13] One even characterized it as a "refuge of the inexperienced and incompetent sonographer." Others simply stated that they did not do a less than complete examination in their units. Only five of 11 radiologists and 13 of 25 obstetricians gave different responses for screening and targeted ultrasonography. Therefore the data for screening sonography should be regarded cautiously.

From the results of the survey (Table I) it is clear that estimates vary widely with respect to the proportion of fetal malformations that may be suspected or diagnosed ultrasonographically. The average estimate is about two thirds by a screening approach and about seven eighths by the more detailed targeted technique. The reported charges varied even more widely, as much as sixfold ($110 to $663) for the advanced study.

Hypothetical considerations. The final segment of this portion of the symposium involves a theoretical approach to what might be possible by antenatal diagnosis of fetal malformation. To make such an analysis, it is necessary to know the relative frequency of various birth defects. The report by Heinonen et al.,[14] which is based on the Collaborative Perinatal Project and published in 1977, represents one source of such information. Among the 50,282 pregnant women en-

rolled, the prevalence of malformed infants was 6.5% and of total malformations 8.8%. Slightly more than half of the malformations were considered major, defined as potentially life-threatening or representing major cosmetic defects. Table II lists the occurrence rates of various defects grouped by organ system.

Musculoskeletal system. The most common defects, in order, were clubfoot, polydactyly-syndactyly, absent or hypoplastic muscle or limb, and dislocated hip. Ultrasonographic diagnosis is questionable and moreover the defects are generally amenable to surgical treatment and correction. Thus ultrasonography has little to offer.

Genitourinary system. Hypospadias, by far the most common defect in this group, is not diagnosable by ultrasonography and is eminently treatable after birth. Polycystic kidneys may be diagnosed ultrasonographically, but whether such a diagnosis before 20 weeks' gestation would justify abortion is arguable. Obstructive uropathy can certainly be diagnosed by ultrasonography and theoretically may benefit from treatment in utero, although the experience with urinary shunting has not been encouraging. Thus ultrasonography is a limited value in genitourinary malformations.

Cardiovascular system. The most common malformations (septal defects, valvular stenosis, patent ductus arteriosus) are usually amenable to surgical treatment after birth. Some less common ones (e.g., hypoplastic left heart, malposition of vessels) are more serious, and early identification with subsequent abortion has some appeal. However, conventional ultrasonography is of limited value in identifying the fetus with congenital heart defects. Without unusual findings such as hydrops or cardiac arrythmia, it is not likely to be helpful. Approaches that include fetal echocardiography offer considerably more potential for diagnosis. However, fetal echocardiography requires special expertise of a type currently found in very few centers.

Gastrointestinal system. The two most common conditions, pyloric stenosis and anal atresia, are not diagnosed by ultrasonography and are easily treated after birth. Less common defects such as omphalocele and gastroschisis, tracheoesophageal fistula, and small bowel atresia can be diagnosed ultrasonographically; such an antenatal diagnosis is likely to be helpful in planning delivery and neonatal care. Additionally, recognition of omphalocele should prompt karyotyping because of the high association of omphalocele and aneuploidy.

Central nervous system. Microcephaly and hydrocephalus are the two most common defects. The former is not generally amenable to ultrasonographic diagnosis, at least early enough for elective abortion. Hydrocephalus is diagnosable by ultrasonography and is, potentially at least, amenable to treatment in utero; however, the experience with shunting procedures has been mixed.

Table II. Prevalence of malformations

System	*Rate (per 1000)*
Musculoskeletal	14.3
Genitourinary	9.2
Cardiovascular	8.0
Gastrointestinal	6.0
Central nervous	5.3
Respiratory	4.3
Syndromes	3.5
Eye and ear	2.4

Data of Heinonen et al.[14]

Table III. Ultrasonographic signs in Down syndrome

	Sensitivity (%)	*Specificity (%)*
Femur length shortening		
Perella et al.[17]	42	88
Dicke et al.[18]	15	90
Cuckle et al.[19]	11	98.6
Grist et al.[20]	50	93.5
Hill et al.[21]	50	85
Biparietal diameter/femur length		
Dicke et al.[18]	18	85
Hill et al.[21]	36.4	93.4
Lockwood et al.[23]	51-70	93-95
Shah et al.[23]	18	94

Respiratory system. The most common defect, cleft lip or palate, conceivably can be recognized ultrasonographically but probably should not lead to abortion. Diagnosis of lung hypoplasia by measuring thoracic diameters has been disappointing; besides, the condition in its severe form is universally fatal. Diaphragmatic hernia can be diagnosed and it has been suggested recently that fetal surgery may offer some benefit.

Syndromes. The most common of these conditions is Down syndrome, and it represents something of a paradigm. Ultrasonographic signs (decreased femur length and increased nuchal skinfold thickness) were reported initially to have a sensitivity of 75% and a specificity of 98%.[15] However, an editorial[16] accompanying this initial report urged physicians "to resist the temptation to introduce ultrasound screening for Down's syndrome into widespread practice before these findings are confirmed by others." How prescient, for subsequent reports (Table III) have yielded widely varying results, especially with respect to sensitivity. Some means of screening for Down syndrome and other aneuploides is much needed and is the focus of considerable effort. However, it seems unlikely that ultrasonography can ever be cost-effective for this purpose. Moreover, it is hard to imagine that it will ever replace karyotyping as the means of diagnosis.

Eye and ear. Cataract, the most common defect in this class, is neither amenable to ultrasonographic diagnosis nor an indication for abortion. Anophthalmia and microphthalmia can be detected by careful ultrasonographic examination, but diagnosis would not likely alter management.

Comment

Although there has been much interest in ultrasonography in the screening and detection of congenital malformation, careful and objective review makes it difficult to generate much enthusiasm for the subject. As a screening test, it fails because of a generally abysmal sensitivity, although specificity is good. Screening tests must stand or fall primarily on the basis of their ability to detect the disease when it is present.

When used for detection of malformations suspected because of history or screening ultrasonography, the targeted examination looks better. Careful study by a skilled ultrasonographer can identify many—though by no means all—malformations. However, this is a higher, labor-intensive process and therefore extremely expensive. If, as Sabbagha et al.[8] stated, an average of one hour is spent in the assessment, our survey's estimate of $250 to $300 may be lower than it should be. Clearly, an appropriate cost-benefit analysis needs to be done regarding this question.

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The current status of maternal and fetal blood flow velocimetry

J.A. Low, MD
Kingston, Ontario, Canada

Significant progress has been achieved in regard to normative data for uteroplacental and fetal blood flow velocity and the factors which may affect these observations. Associations have been demonstrated between abnormal indices of maternal and fetal blood flow velocity and pregnancy-induced hypertension, fetal growth retardation, fetal blood gas measures, and intrapartum clinical fetal distress. However, in general, the predictive power is modest and no evidence has yet been provided to indicate that these measures can be used as a screening test in the general obstetric population. Continuing research is highly desirable. At the present time, the use of Doppler assessment of blood flow in pregnancy should be limited to the investigational setting. (Am J Obstet Gynecol 1991;164:1049-63.)

Key words: Blood flow velocimetry, hypertensive disorders, growth retardation, fetal asphyxia

It is well recognized that compromised uteroplacental or fetal circulation may play an important role in hypertensive disorders in pregnancy, intrauterine growth retardation, and fetal asphyxia. In the past, the clinical assessment of maternal and fetal hemodynamics has not been possible. However, the development of Doppler ultrasonographic technology, particularly during the last 10 years, has provided an opportunity to obtain both qualitative and quantitative assessment of maternal and fetal hemodynamics in a noninvasive manner.

A National Institutes of Health consensus conference in September 1986 reviewed the development of Doppler ultrasonography. It was emphasized that even if altered velocity was confirmed as being able to detect fetal compromise with sensitivity and specificity, additional clinical trials would be necessary to determine whether intervention that is based on Doppler findings actually improved outcome. One conclusion of the conference stated, "It is desirable to limit the use of Doppler assessment in pregnancy to the investigational setting until the appropriate clinical trials are concluded."

The objective of this review is to examine the current understanding of measures of maternal and fetal blood flow velocity and the role of these measures in the assessment of particular maternal and fetal complication.

Measurement of maternal and fetal blood flow

Accurate measures of blood flow would be ideal. Estimates of uteroplacental blood flow have been obtained from the uterine artery with a transvaginal probe.[1] This study reported an approximate fourfold increase of blood flow in the uterine artery at term in relation to prepregnancy values.

The results of fetal blood flow studies in the umbilical vein and aorta are outlined in Table I. Measures of fetal blood flow per kilogram of body weight per minute during the last trimester have been reasonably comparable. The proportion of umbilical vein to aortic blood flow has ranged from 50% to 64%. The ratio of umbilical to aortic flow remains constant between 26 weeks and term.[12]

However, there are several sources of error in the measurement of maternal and fetal blood flow. The calculation of mean blood flow requires an accurate measure of blood flow velocity. The formula for the calculation of mean blood flow velocity from the waveform may vary, and errors of the order of 20% may occur in the estimation of the angle of insonation.[6] Small errors in the measurement of vessel diameter are compounded in the calculation of blood flow.

Estimates of error of <10% to 15% have been reported with special instrumentation and careful attention to the sources of error.[13] Thus there is a role for measures of blood flow in clinical research in dedicated centers. However, errors up to 50% are perfectly realistic when commercially available sector scanners with minimal signal processing are used.[14] In addition, wide SDs of the available reference data limit the predictive value of measures of blood flow.[15] Thus, because of these methodologic problems, blood flow measurements have been largely abandoned in clinical studies.

Measurement and maternal and fetal blood flow velocity

Maternal and fetal blood flow velocity indices avoid the major sources of error in the measurement of blood

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Reprint requests: J.A. Low, MD, Department of Obstetrics and Gynecology, Queen's University, Kingston, Ontario, Canada K7L 3N6.
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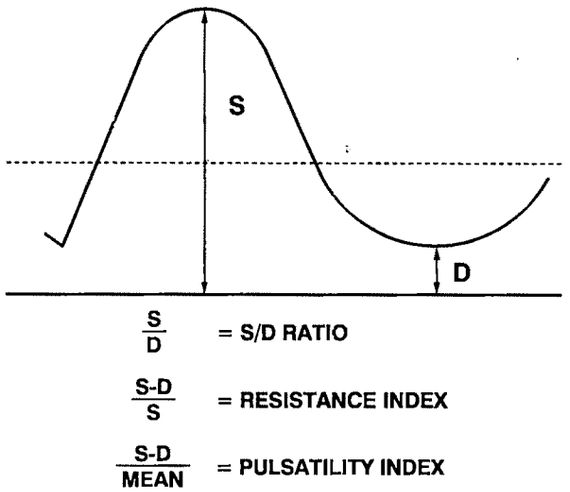


Fig. 1. Indices of maternal and fetal blood flow velocity.

flow. These indices are independent of the angle of insonation and do not require a measurement of the diameter of the vessel. There is a close correlation in normal pregnancies between the three indices used in most clinical studies[16] (Fig. 1). The systolic/diastolic ratio[17] and the Pourcelot resistance index[18] are the simplest indices to measure. However, errors increase with absent end-diastolic velocity so that systolic/diastolic ratios approach infinity or the resistance index approaches one. The pulsatility index[19] requires digitizing the entire waveform, making it the least convenient index. Variations of the pulsatility index reported by different centers may reflect differing methods of calculating mean blood flow velocity in commercially available analyzers.[20, 21] Although the pulsatility index incorporates additional information in regard to the waveform, the extra computation does not contribute significantly to current clinical interpretation.[22, 23]

Signals of the distinctive maternal and fetal waveforms can be obtained by either pulsed wave or continuous wave Doppler equipment. No significant difference has been reported for waveform measures obtained by these two techniques.[24] However, a better correlation between continuous wave and pulsed wave measures has been observed in the umbilical artery rather than in the uterine artery.[25]

Interobserver and intraobserver variation has been specifically examined by several investigators.[26-29] No significant differences have been reported with, in general, a coefficient of variation <10%.

A waveform analysis yields information relating to the condition of the proximal circulation and the impedance of the distal vascular bed. These relationships have been examined in the laboratory. A computerized model simulating uteroplacental waveforms has demonstrated that increasing uteroplacental vascular resistance caused an increase of the systolic/diastolic ratio or the pulsatility index with the appearance of a dicrotic notch. Alterations of blood pressure in this model influenced mean blood flow velocity but had little effect on these indices.[30] In the laboratory, embolization of the fetal side of the placental circulation in the fetal lamb results in a decrease of end-diastolic velocity to zero, with a resultant increase of the umbilical artery systolic/diastolic ratio.[31, 32] A similar change has been demonstrated in the exteriorized fetal lamb by constriction of the umbilical vein, resulting in retrograde end-diastolic flow in the umbilical artery and aorta.[33]

Thus in clinical studies waveforms with a high flow in diastole accompany a low downstream impedance, whereas waveforms with little flow, or reversed flow during diastole, are seen when impedance downstream is high. There is an increasing body of evidence to indicate that elevated indices of blood flow velocity in the fetal circulation reflect a specific microvascular pathologic condition in the tertiary villi of the placenta. Examination of the placenta from patients with elevated systolic/diastolic ratios demonstrates a striking reduction of the number of small muscular arteries in the tertiary villi.[34-36]

Reference values for maternal and fetal blood flow velocity. The uteroplacental circulation, including the uterine, arcuate, radial, and spiral arteries, is complex. This circulation undergoes remarkable changes during pregnancy to accommodate the growing fetus. The trophoblast invades the spiral arterioles, replacing the musculoelastic component of the vessel wall and reducing peripheral resistance to permit this accommodation.

The main stem uterine arteries may be identified with a duplex sector scanner and a vaginal probe.[37] The uterine arteries also may be examined abdominally, with identification of the distinctive uterine artery waveform.[38] Alternatively, the arcuate arteries may be examined in a placental or nonplacental site. However, abdominal observations of the radial or arcuate arteries represent one vessel in a complex circulation, so that reproducible identification of an arcuate artery is difficult.

Reference values of uteroplacental blood flow velocity from uncomplicated pregnancies reported by a number of investigators are outlined in Table II. Indices of uteroplacental blood flow velocity decrease in early pregnancy until 20 to 26 weeks' gestation and then remain stable to term. The high end-diastolic blood flow velocity and low ratios during the last half of pregnancy reflect the low peripheral vascular resistance of the placenta.

The sampling site must be known. Indices of blood flow velocity tend to be lower in the arcuate artery than

Table I. Measures of fetal blood flow in umbilical vein and dorsal aorta during last half of pregnancy

Year	Series	Study population (N)	Gestational age (wk)	Blood flow	
				Umbilical vein (ml/kg/min)	Aorta (ml/kg/min)
1980	Eik-Nes et al.[2]	26	32-41	110	191
1981	Gill et al.[3]	27	26-35	120	
		20	35-46	106	
1981	Jouppila et al.[4]	101	30-36	108	
1982	Kurjak and Rajhvajn[5]	63	30-41	107	
1983	Griffin et al.[6]	45	24-42	122	246
1984	Van Lierde et al.[7]	20	37-40	117	216
1984	Marsal et al.[8]	64	27-40		240
1985	Erskine and Ritchie[9]	15	28-40	125	206
1986	Lingman and Marsal[10]	21	28	139	241
			40	65	213
1987	Gerson et al.[11]	209	20	153	
			30	131	
			40	108	

Table II. Indices of uteroplacental blood flow velocity between 16 weeks and term

Year	Series	Study population (N)	Protocol		Gestational age (wk)						
			Site	Index	16	20	24	28	32	36	40
1985	Trudinger et al.[39]	12	Abd.	S/D				3.7			3.0
1986	Schulman et al.[38]	79	Abd.	S/D	2.9	2.3	2.2	1.9	2.0	2.0	2.0
			Vag.	S/D	4.1	3.0	2.7	2.3	2.1	2.0	2.0
1988	Deutinger et al.[37]	71	Vag.	S/D	3.0	3.0	2.4	2.6	2.0	2.0	2.2
1990	Thaler et al.[1]	24	Vag.	S/D	3.2	2.8		2.5	2.3	2.3	
1988	Pearce et al.[40]	40	Abd.	RI	0.55		0.50				0.42
			NP-P	RI	0.48		0.42				0.40

Abd., Abdominal; *Vag.*, vaginal; *NP*, nonplacental; *P*, placental; *S/D*, systolic/diastolic ratio; *RI*, resistance index.

in the uterine artery. Indices are lower in placental than in nonplacental sites.[41, 42] These differences are particularly striking in the second trimester, when differences up to 50% may occur between observations from nonplacental and placental sites in the arcuate and uterine arteries.[42]

Reference values of fetal aortic blood flow velocity from uncomplicated pregnancies as reported by a number of investigators are outlined in Table III. The indices of vascular resistance in the fetal aorta remain unchanged during the last half of pregnancy. The relatively high indices reflect the low end-diastolic blood flow velocity due to the vascular resistance of the nonplacental circulation. Since the mean blood flow velocity in the fetal aorta is constant, the increase in total blood flow is due to the increase in the diameter of the fetal aorta from 3 or 4 mm to 7 or 8 mm during the last half of pregnancy.[12, 47]

Umbilical artery blood flow velocity indices are lower at the placental than the fetal end of the cord.[48-50] Thus the usual protocol is to record blood flow velocity observations from the placental end of the cord. Reference values for umbilical artery blood flow velocity for uncomplicated pregnancies reported by a number of investigators are outlined in Table III. Indices prior to 20 weeks' gestation are of limited value because of either low or absent end-diastolic velocity. Although there is some variation in the reported mean values for each gestational age, there was a decrease of all indices during the last half of pregnancy. A similar pattern has been observed in studies of twin pregnancies.[38, 60] The wide 95% confidence limits, particularly in the second trimester, represent a limitation in the identification of compromised pregnancies at this stage.[51]

Reference values for the fetal cerebral circulation blood flow velocity from uncomplicated pregnancies reported by a number of investigators are outlined in Table III. Observations have been made in the common carotid artery,[45] the internal carotid artery,[41, 62] and the middle cerebral artery.[46, 61] Common carotid end-diastolic velocity was not observed until 32 weeks' gestation because of the vascular impedance of the external carotid vascular bed. Middle cerebral artery end-diastolic blood flow velocity is absent or low with corresponding high indices until 28 to 32 weeks' gestation. Thereafter there is a significant decrease of all indices to term,[46, 61, 62] reflecting decreased vascular re-

Table III. Indices of blood flow velocity in fetal aorta, umbilical vein, and fetal cerebral circulation during last half of pregnancy

Year	Series	Protocol		Gestational age (wk)					
		Population (N)	Index	20	24	28	32	36	40
Fetal aorta									
1986	Wladimiroff et al.[44]	40	PI			1.8	1.8	1.8	1.7
1988	Bilardo et al.[45]	70	PI		2.1		2.2		2.1
1989	Arstrom et al.[46]	24	PI		1.8				1.9
			S/D		5.4				5.5
Umbilical artery									
1984	Schulman et al.[51]	89	S/D	3.3	3.0	2.8	2.8	2.5	2.3
1987	Gerson et al.[52]	171	S/D	4.1	3.6	3.1	2.6	2.1	1.7
1988	Thompson et al.[53]	35	S/D	4.0	3.5	3.2	2.8	2.2	2.1
1988	Al-Ghazali et al.[54]	271	S/D	4.2	3.8	3.5	3.1	3.0	2.2
1989	Hendricks et al.[55]	590	S/D	4.3	3.8	3.5	3.0	2.6	2.4
1988	Gudmundson and Marsal[56]	125	S/D	3.9		3.5	2.8		2.3
1989	Astrom et al.[46]	22	S/D	3.3		3.0	2.7		2.2
1984	Fitzgerald et al.[57]	134	RI	0.75	0.73	0.71	0.68	0.63	0.57
1988	Thompson et al.[53]	35	RI	0.75	0.71	0.68	0.63	0.55	0.52
1989	Hendricks et al.[55]	590	RI	0.74	0.72	0.70	0.65	0.60	0.54
1984	Reuwer et al.[58]	23	PI	2.2	1.5	1.0	0.8	0.80	0.80
1985	Erskine et al.[59]	15	PI			1.0	0.8		0.65
1986	Wladimiroff et al.[44]	42	PI			1.3	1.0	0.80	0.7
1988	Pearce et al.[40]		PI	2.2	1.5	1.0	0.8	0.8	0.8
1988	Gudmundson et al.[56]	125	PI	1.3		1.15	1.0		0.8
1989	Arstrom et al.[46]	22	PI	1.4		1.15	1.1		0.87
Cerebral artery									
1987	Woo et al.[61]	14	S/D		6.9				4.2
1989	Arstrom et al.[46]	22	S/D			8.4			3.8
1987	Kirkinen et al.[62]	83	RI			0.84	0.80		0.72
1989	Arstrom et al.[46]	22	RI			0.87	0.84		0.72
1986	Wladimiroff et al.[44]	42	PI			1.6	1.6	1.5	1.5
1988	Bilardo et al.[45]	70	PI*			3.8	3.6	3.2	2.2

PI, Pulsatility index; *S/D,* systolic/diastolic ratio; *RI,* resistance index.
*Common carotid.

sistance in the cerebral circulation. It has been speculated that these changes are a response to changing fetal blood gases with advancing gestational age.[45]

Factors affecting maternal and fetal blood flow velocity. There is an inverse relationship between fetal heart rate and blood flow velocity in the umbilical artery, fetal aorta, and carotid artery. A significant negative correlation has been observed between fetal heart rate and umbilical artery systolic/diastolic ratios or pulsatility index in the antepartum period[63-65] and in the intrapartum period.[63, 66] A similar correlation has been observed in the fetal aorta and carotid artery.[64] Although the response may be variable, spontaneous or induced fetal heart rate accelerations have been associated with decreased umbilical artery systolic/diastolic ratios.[66, 67] However, no significant correlation was observed for the normal range of fetal heart rates between 120 and 160 beats/min and umbilical artery systolic/diastolic ratios.[68] Thus, although there is an association between fetal heart rate and blood flow velocity, at this time it is not considered sufficient to influence clinical interpretation of fetal blood flow velocity waveforms.

Several factors affecting maternal and fetal heart rate have been examined. Smoking a single cigarette is associated with an increase of maternal heart rate and blood pressure; however, no change in uteroplacental blood flow velocity was observed.[69] Maternal smoking is also associated with an increase in fetal heart rate.[70-72] This fetal tachycardia was associated with an increase in blood flow velocity and a transient decrease of the pulsatility index in the fetal aorta and umbilical artery.[70, 71] Graded maternal exercise is associated with an increase in maternal heart rate and blood pressure and fetal heart rate. However, no consistent changes in uteroplacental or umbilical artery systolic/diastolic ratios have been observed with exercise.[72, 73]

Blood viscosity may affect blood flow velocity. However, in current clinical studies no association has been observed between maternal blood viscosity and uteroplacental blood flow velocity or between fetal blood viscosity and umbilical artery blood flow velocity.[74-76]

Laboratory studies have demonstrated that behavioral state will affect regional blood flow, particularly to the brain.[77, 78] Studies at term[79] and at 27 to 28 weeks' gestation[80] have demonstrated a reduction in the pulsatility index in the fetal internal carotid artery and the fetal aorta during the active sleep state in relation to the quiet sleep state.

The effects of drugs that are used in the management

Table IV. Incidence of abnormal uteroplacental index values in obstetric patients with hypertension

Year	Series	Gestational age at observation (wk)	Hypertension		Abnormal uteroplacental index (%)
			Class	No.	
1985	Trudinger et al.[39]	>20	Mixed	25	44
1986	Campbell et al.[89]	18-20	PIH	15	64
1987	Arduini et al.[90]	18-20	PIH	22	64
1987	Ducey et al.[91]	>26	Chronic hypertension	43	12
			PIH	34	26
			Toxemia	51	57

PIH, Pregnancy-induced hypertension.

of preterm labor on blood velocity have been examined. Oral nifedipine had no apparent effect on uteroplacental or fetal blood flow velocity.[81] Similarly, magnesium sulfate was associated with no change in maternal or fetal blood flow velocity.[82] However, ritodrine, with an increase in maternal and fetal heart rate, was associated with a progressive decrease in both uteroplacental and umbilical artery systolic/diastolic ratios.[82] Maternal use of indomethacin was not associated with a significant change in umbilical artery systolic/diastolic ratio.[83] However, in the presence of ductal constriction and tricuspid insufficiency, indomethacin may be associated with a decrease in the pulsatility index in the middle cerebral artery.[84]

Spontaneous uterine contractions of labor result in a progressive decrease of uteroplacental end-diastolic blood flow velocity. The intensity of the contraction correlates with the decrease of the end-diastolic velocity.[85] In one study[86] the end-diastolic velocity reached zero with intrauterine pressures >35 mm Hg. Umbilical artery systolic/diastolic ratios are unchanged during labor in the absence of fetal heart rate decelerations. Epidural analgesia during labor or anesthesia at delivery has no apparent effect on uteroplacental or umbilical artery blood flow velocity.[87, 88]

Prediction of hypertension in pregnancy

It has been proposed that abnormal uteroplacental waveforms may identify obstetric patients at risk for hypertension in pregnancy.[89] Evidence to demonstrate this association is outlined in Table IV. Two studies examined hypertension in pregnancy from various causes. Trudinger et al.[39] observed abnormal uteroplacental waveforms in 11 of 25 patients with hypertension. Ducey et al.[91] reported the results for 136 patients with hypertension. The percentages of patients with a uteroplacental systolic/diastolic ratio >2.6 after 26 weeks were as follows: chronic hypertension, 12%; pregnancy-induced hypertension, 26%; and toxemia of pregnancy, 56%.

Two studies examined the predictive value of uteroplacental blood flow velocity at 18 to 20 weeks' gestation. Campbell et al.[89] examined 126 consecutive obstetric patients, 15 of whom had pregnancy-induced hypertension. Ten patients (64%) had a uteroplacental resistance index >0.58. The authors concluded that an abnormal arcuate artery blood flow velocity observation could be a valuable predictor of pregnancy-induced hypertension. Arduini et al.[90] reviewed 60 pregnancies at risk, 22 of which subsequently developed pregnancy-induced hypertension. The obstetric patients with hypertension had a higher uteroplacental resistance index than those with a normal blood pressure. The predictive characteristics of an abnormal uteroplacental resistance index were: sensitivity, 64%; specificity, 84%; positive predictive value, 70%; and negative predictive value, 80%.

One study did not demonstrate an association between uteroplacental blood flow velocity and pregnancy-induced hypertension. Hanretty et al.[92] in a matched cohort study compared 32 patients with untreated pregnancy-induced hypertension with 32 patients with normal blood pressure. Serial Doppler observations were obtained, and the lowest resistance index was used for analysis. There was no significant difference between the uteroplacental pulsatility index of the hypertensive group and the control group. High and low pulsatility indices were found in both groups, with occasional divergent indices occurring in the same patient.

Prediction of fetal growth retardation

Three studies have examined the relationship between the final uteroplacental blood flow velocity observation before delivery and fetal growth retardation at delivery. Trudinger et al.[39] reported low end-diastolic blood flow velocity in 15 of 25 growth-retarded newborn infants and suggested that a change in uterine perfusion was a primary event in some cases of growth retardation. McCowan et al.[93] reported reduced end-diastolic blood flow velocity in seven of 12 growth-retarded newborn infants. Six of these seven cases occurred in pregnancies complicated by severe toxemia. However, Gudmunsson et al.,[56] in a study of 129 selected pregnancies of whom 66 delivered growth-retarded infants, observed no significant difference in the mean resistance index for growth-retarded infants in relation to normal infants.

Three studies reported data assessing the predictive value of uteroplacental blood flow velocity in growth

Table V. Predictive value of uteroplacental blood flow velocity for growth retardation in newborns

Year	Series	Study population			Observation protocol	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
		Total No.	SGA						
			n	%					
1985	Trudinger et al.[39]	91	25	27	Final	60	80	53	84
1989	Chambers et al.[94]	145	85	59	Single	29	80	66	44
1990	Jacobson et al.[95]	93	17	18	20 and 24 wk	70	64	33	89

SGA, Small for gestational age.

retardation (Table V). The design was different in each study. Trudinger et al.,[39] using the final uteroplacental blood flow velocity observation before delivery, reported a sensitivity of 60% with a positive predictive value of 53%. Chambers et al.[94] examined the predictive value of a single uteroplacental blood flow observation during the last trimester in 145 selected patients, 85 of whom were delivered of growth-retarded infants. There was no significant difference in the mean resistance index for the growth-retarded infants in relation to the normal infants. The resultant sensitivity of this single observation was low, 29%, with a positive predictive value of 66%. Jacobson et al.[95] examined the predictive value of uteroplacental blood flow velocity measures at 20 and 24 weeks' gestation to growth retardation at delivery in 93 selected pregnancies. There was evidence of an association between a higher mean resistance index at both 20 and 24 weeks and growth retardation at delivery. A reasonable sensitivity, 70%, was achieved; however, the positive predictive value was 33%. The authors concluded that the high false-positive rate would limit the clinical value of such observations.

A number of descriptive studies of fetal blood flow velocity in the aorta[96, 97] or the umbilical artery[59, 94, 98-102] in growth-retarded infants have demonstrated an association between abnormal indices of blood flow velocity and growth retardation. The incidence of abnormal fetal blood flow velocity in the growth-retarded infants in these studies ranged from 60% to 83%.

There have been differences in the design of a number of studies which have examined the predictive value of umbilical artery blood flow velocity for fetal growth retardation (Table VI). Most study populations have been of selected-risk pregnancies with two studies of unselected pregnancies. The umbilical artery blood flow velocity observations have been either the last observation before delivery, serial observations during the third trimester, or observations at specific gestational ages.

Giles et al.[103] examined the relationship between the final umbilical artery blood flow velocity observation before delivery and growth retardation. All 10 growth-retarded infants had an abnormal umbilical artery systolic/diastolic ratio.

The relationship between serial umbilical artery blood flow velocity observations in the third trimester and fetal growth retardation has been examined in four studies. Fleischer et al.[104] observed a significantly higher umbilical artery systolic/diastolic ratio in newborn infants in the first quartile in relation to those with a weight/gestational age percentile greater than the first quartile. The sensitivity of an abnormal umbilical artery systolic ratio for growth retardation was 75%. Three studies in selected pregnancies reported a less favorable association, with a sensitivity of <50%. Divon et al.[105] found that ultrasonographic estimation of fetal weight (sensitivity, 87%) was a better predictor than the umbilical artery systolic/diastolic ratio. Berkowitz et al.[106] observed that the predictive value of the final umbilical artery blood flow velocity observation was superior to observations earlier in the third trimester. Dempster et al.[107] noted the large number of false-positive results, in addition to the limited sensitivity of umbilical artery blood flow velocity during the third trimester.

Two studies[108, 109] have examined umbilical artery blood flow velocity at specific gestational ages in selected pregnancies. There was a significant association between increased umbilical artery systolic/diastolic ratios or pulsatility index and growth retardation. The sensitivities were 53% and 65%, and the positive predictive values were 66% and 50%.

Two studies have examined umbilical artery blood flow velocity in large unselected populations. Sijmons et al.[110] reported a study of 400 unselected pregnancies in a tertiary care center with an overall 20% incidence of growth retardation. Umbilical artery blood flow velocity observations were performed at 28 and 34 weeks. The prediction for growth retardation was poor at both gestational ages. It was not improved by combining the two observations. Beattie and Dornan[111] reported a study of 2097 unselected singleton pregnancies. Um-

Table VI. Predictive value of fetal blood flow velocity for fetal growth retardation

Year	Series	Study population			Study protocol		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
		No.	SGA		Time of observation	Index				
			n	%						
1986	Giles et al.[103]	42*	10	24	Final	S/D	100	88	71	100
1985	Fleischer et al.[104]	137*	23	16	3rd Tri.	S/D	78	83	49	95
1987	Mulders et al.[109]	48*	15	31	34 wk	S/D	53	88	66	80
1988	Divon et al.[105]	127*	45	35	3rd Tri.	S/D	49	94	81	77
1988	Berkowitz et al.[106]	168*	42	25	3rd Tri.	S/D	45	89	57	
1989	Dempster et al.[107]	205*	82	40	3rd Tri.	S/D	41	82	61	68
1987	Arduini et al.[108]	75*	23	31	26-28	PI	61	73	50	81
1989	Sjmons et al.[110]	400†	88	22	28 wk	PI	17	95	50	79
					34 wk	PI	22	94	53	81
1989	Beattie and Dornan[11]	2097†			28 wk	PI	28	89	11	97
					34 wk	PI	32	89	12	97
					38 wk	PI	31	86	9	97

S/D, Systolic/diastolic ratio; *PI*, pulsatile index; *3rd Tri.*, serial measures throughout third trimester.
*High-risk study population.
†Random study population.

bilical artery blood flow velocity observations were obtained at 28, 34, and 38 weeks. The prediction of growth retardation was poor at each gestational age. The prediction, on the basis of a low ponderal index in both studies, was of the same order as the prediction of infants less than the 10th centile for weight/gestational age.

Preliminary experience has demonstrated that recording fetal carotid artery blood flow velocity may be difficult. Wladimiroff et al.[112] obtained satisfactory waveforms in 74% of pregnancies studied. Technical difficulties included maternal obesity, fetal head in the occipitoanterior position, and fetal movement. Two studies have reported a significantly lower carotid artery pulsatility index in the growth-retarded fetus than in the normally grown one.[108, 112] This decreased vascular resistance is postulated to reflect the redistribution of blood flow to the brain in the stressed fetus. However, Satoh et al.[101] observed a decrease in the carotid artery pulsatility index only in the growth-retarded fetus with fetal heart rate decelerations. One study[102] did not find a significant difference in the carotid artery pulsatility index in the growth-retarded fetus as compared with the normally grown fetus.

Association between fetal blood flow velocity and fetal blood gas values

The introduction of cordocentesis provided an opportunity to examine the relationship between fetal blood flow velocity and fetal blood gas and acid-base characteristics. Three studies have examined these relationships (Table VII). Two studies[113, 114] have demonstrated a significant correlation between increasing mean blood flow velocity in the fetal aorta and favorable umbilical vein oxygen tension, carbon dioxide tension, and pH. Similarly, a correlation has been demonstrated between decreasing pulsatility index in the umbilical artery[115] and aorta[114] and favorable umbilical vein blood gases.

Simonazzi et al.[116] examined the relationship between the internal carotid artery pulsatility index and blood gases and acid-base measures from the fetal scalp in 17 normal term pregnancies. There was a positive correlation between the pulsatility index in the internal carotid artery and fetal oxygen tension, suggesting that fetal oxygen tension has a regulatory role in cerebrovascular resistance under physiologic conditions.

Nicolaides et al.,[117] in a study of 59 growth-retarded fetuses with absent umbilical artery end-diastolic blood flow velocity, sampled the umbilical vein by cordocentesis. Hypoxia (oxygen tension <2 SD below the mean) was observed in 80%, and acidosis (pH <2 SD below the mean) was observed in 46%.

Some evidence of a relationship between fetal blood flow velocity and umbilical vein and artery blood gases at delivery has been reported. Ferrazzi et al.[115] in a study of 12 fetuses at delivery demonstrated a good correlation between umbilical artery blood flow velocity and umbilical vein and artery oxygen tension, carbon dioxide tension, pH, and lactate. Two studies have demonstrated a relationship between absent umbilical artery end-diastolic velocity and fetal blood gases at delivery. Brar et al.[118] observed an increased incidence of acidosis (umbilical artery pH <7.2). Tyrrell et al.[119] observed an increased incidence of hypoxemia and acidosis (umbilical vein <7.25).

However, the relationship of fetal blood flow velocity to fetal blood gases at delivery has not been consistent, with four studies failing to demonstrate a significant association. Laurin et al.[120] did not find a relationship

Table VII. Correlation between fetal blood flow velocity and umbilical vein blood gas values obtained by cordocentesis

Year	Series	Population (No.)	Protocol		Blood gas sample	Correlations		
			Site	Index		Po_2	Pco_2	pH
1986	Soothill et al.[113]	29	Ao.	MBFV	Umb. v	0.73	−0.54	0.58
1990	Bilardo et al.[114]	51	Ao.	MBFV	Umb. v	0.51	−0.59	0.54
1988	Ferrazzi et al.[115]	6	Umb. a	PI	Umb. v		0.86	−0.98
1990	Bilardo et al.[114]	51	Ao.	PI	Umb. v	−0.47	0.57	−0.57

Ao., Aorta; *umb. a*, umbilical artery; *umb. v*, umbilical vein; *MBFV*, mean blood flow velocity; *PI*, pulsatile index.

between aortic blood flow velocity and cord blood acidosis at delivery. McCowan et al.[121] in 15 severely growth-retarded fetuses reported no association between umbilical artery pulsatility index and cord blood gases at delivery. Divon et al.[122] reported similar umbilical artery pH in a matched cohort study of fetuses with normal and abnormal umbilical artery systolic/diastolic ratios. Lowery et al.[123] observed the same incidence of low pH in fetuses with normal and abnormal umbilical artery systolic/diastolic ratios.

Prediction of clinical fetal distress

A wide range of studies has addressed the question of predicting clinical fetal distress. Study populations have included pregnancies producing growth-retarded fetuses, postterm pregnancies, pregnancies at risk because of one or more maternal, obstetric, or fetal complication, or general obstetric populations. Maternal or fetal blood flow velocity observations have been obtained at varied times between 18 weeks and early labor. The outcome measures have usually been clinical fetal distress leading to intervention by either operative vaginal delivery or cesarean section. Diagnosis of fetal distress has been based on some combination of meconium in the amniotic fluid or abnormality of fetal heart rate, including decreased baseline variability or fetal heart rate decelerations.

Two studies of selected-risk pregnancies have provided data on the association of uteroplacental blood flow velocity and fetal distress in labor. Schulman et al.[124] in a study of 71 selected pregnancies observed a higher incidence of fetal distress in pregnancies with abnormal uteroplacental systolic/diastolic ratios (27%), in relation to pregnancies with normal systolic/diastolic ratios (5%). On the other hand, Brar et al.[125] in a study of 92 selected pregnancies observed no association between uteroplacental systolic/diastolic ratios and fetal distress.

Four studies of selected-risk pregnancies have provided data on the association between fetal blood flow velocity and fetal distress in labor. Three studies have demonstrated a significant association between absent end-diastolic velocity in the aorta[120, 126] and umbilical artery[127] and fetal distress in labor. Brar et al.[128] presented data to indicate that measurement of umbilical artery blood flow velocity can identify the late decelerations observed before or in early labor that are predictive of fetal distress.

Two studies of growth retardation have examined the association between umbilical artery blood flow velocity and fetal distress in labor. Rochelson et al.[129] in a study of 54 pregnancies producing growth-retarded infants observed fetal distress in 20 of 38 (53%) with abnormal umbilical artery systolic/diastolic ratios. Brar et al.[118] in a study of eight pregnancies with growth retardation in which reversed end-diastolic velocity reflected the severe end of the placental vascular resistance spectrum observed fetal distress during labor in six of the eight cases (75%).

Four studies of postterm pregnancies have examined the association between uteroplacental and fetal umbilical artery blood flow velocity and fetal distress in labor. Rightmire et al.[130] observed a small increase in umbilical artery pulsatility index in the postterm fetus with fetal distress. However, three studies have found no evidence that uteroplacental[131] or umbilical artery blood flow velocity[131-133] can identify the postterm fetus at risk of fetal distress during labor. The nonstress test proved to be a better predictor of fetal distress in these pregnancies.[131, 133] These authors concluded that maternal or fetal blood flow velocity studies are unlikely to be useful in the assessment of fetal status in prolonged pregnancy.

Five studies of selected-risk pregnancies have provided data on the predictive value of fetal blood flow velocity for fetal distress during labor (Table VIII). Two studies have demonstrated that absent end diastolic blood flow velocity in the fetal aorta[134] and in the umbilical artery[135] may be useful predictors of fetal distress with a sensitivity of 83% and 75%, respectively. However, abnormal umbilical artery blood flow velocities, as defined in these studies, are not as effective as predictors, with sensitivities ranging from 33% to 57%. The limitations of these observations are further emphasized by the low positive predictive values, ranging from 24% to 50%. Several authors note that the ab-

Table VIII. Predictive value of fetal blood flow velocity for fetal distress in labor

Year	Series	Study population (No.)	Fetal distress (%)	Protocol		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
				Site	Index				
1987	Laurin et al.[134]	159*	19	Fet. Ao.	AEDV	83	90	66	96
1987	Rochelson et al.[135]	161*	13	Umb. a	S/D	57	72	24	92
1988	Berkowitz et al.[136]	172*	12	Umb. a	S/D	43	81	24	91
1988	Farmakides et al.[137]	140*	27	Umb. a	S/D	53	76	45	81
1990	Lowery et al.[123]	146*	27	Umb. a	S/D	33	88	50	78
1990	Newnham et al.[139]	469†	9	Umb. a	S/D	12	95	18	91
		443†	9	Umb. a	S/D	15	95	23	92
1989	Feinkind et al.[140]	273†		Umb. a	S/D	29	92	30	92

Fet. Ao., Fetal aorta; *Umb. a*, umbilical artery; *AEDV*, absent end-diastolic velocity; *S/D*, systolic/diastolic ratio.
*Selected pregnancies at risk.
†Random population at 28 weeks.

normal blood flow velocity observations precede other measures of fetal state and as such may prove to be useful complementary observations to the nonstress test and biophysical profile in the prediction of fetal distress.

Two random studies of obstetric populations have provided data on the predictive value of maternal and fetal blood flow velocity for fetal distress during labor. Hanretty et al.[133] examined 357 random pregnancies between 26 and 30 weeks' gestation. Newnham et al.[139] examined 535 random pregnancies at 18, 24, 28, and 34 weeks. Both studies concluded that uteroplacental blood flow velocity measures at these particular gestational ages were of little value in the prediction of clinical fetal distress in labor. Similarly, although there was some evidence of an association between abnormal umbilical artery blood flow velocity at these particular gestational ages and clinical fetal distress during labor, both the sensitivity and positive predictive values are low.

Two random studies of obstetric patients have examined the relationship between umbilical artery blood flow velocity in early labor and clinical fetal distress in labor. Feinkind et al.[140] observed an association between abnormal umbilical artery systolic/diastolic ratios and fetal distress. However, both the sensitivity and positive predictive values were low (Table VIII). Sarno et al.,[141] in a smaller study, found no relationship between abnormal umbilical artery systolic/diastolic ratios in early labor and clinical fetal distress during labor.

Prediction of low Apgar scores at 5 minutes

A number of studies have examined the relationship between fetal blood flow velocity and Apgar scores <7 at 5 minutes. No correlation was reported in four studies.[121, 134, 138, 140]

Data regarding the incidence of Apgar scores <7 at 5 minutes in relation to normal and abnormal fetal blood flow velocity are outlined in Table IX. No significant relationship between abnormal fetal blood flow velocity and low Apgar score at 5 minutes was identified. The only exception was reported by Brar et al.[128] The predictive value of an umbilical artery systolic/diastolic ratio >3.5 in the last trimester was a sensitivity of 53% and a positive predictive value of 38%.

Comment

Many investigators have contributed to the current understanding of normative data for both maternal and fetal blood flow and blood flow velocity and the factors that may affect these observations. However, in spite of this progress, the role of Doppler velocimetry in clinical practice has yet to be determined.

Measurements of maternal and fetal blood flow would be ideal. However, the well-documented sources of error in the measurement of blood flow limits the use of these measures to carefully designed research projects. Thus current clinical studies are examining measures of maternal and fetal blood flow velocity. A statement of procedure is important in the absence of a standardized protocol. The uteroplacental blood flow velocity will differ, particularly in the second trimester, if sampled in the uterine or arcuate arteries at a placental or nonplacental site. Measures in the fetal circulation are most easily achieved in the umbilical artery, preferably from the placental end of the cord. Observations in the fetal aorta and cerebral circulation will not be satisfactory for technical reasons, in some cases, and interpretation of the results may be more difficult because of the rather low end-diastolic velocity in these vessels.

Reference data derived from uncomplicated pregnancies have been obtained for uteroplacental blood flow velocity and fetal blood flow velocity in the umbilical artery, aorta, and cerebral circulation. The absence of end-diastolic blood flow velocity limits the interpretation of uteroplacental, fetal aorta, and umbilical artery blood flow velocity before 20 weeks and fetal

Table IX. Association between abnormal fetal blood flow velocity and Apgar scores <7 at 5 minutes

Year	Series	Population	Protocol site	Index	Total (No.)	Apgar score <7 at 5 min		p Value
						n	%	
1987	Hackett et al.[142]	SGA	Fet. Ao.	EDV	20	1	5	NS
				AEDV	26	7	27	
1987	Rochelson et al.[135]	SHR	Umb. a	S/D <3	109	1	1	NS
				S/D >3	42	4	10	
1988	Berkowitz et al.[136]	SGA	Umb. a	S/D <3	22	0	—	NS
				S/D >3	21	3	14	
1988	Berkowitz et al.[136]	AGA	Umb. a	S/D <3	120	1	1	NS
				S/D >3	26	1	6	
1989	Divon et al.[122]	SHR	Umb. a	S/D N	51	1	2	NS
				S/D Abn.	51	2	4	
1989	Brar et al.[128]	SHR	Umb. a	S/D <3.5	80	5	5	0.05
				S/D >3.5	12	4	38	
1990	Lowery et al.[123]	SHR	Umb. a	S/D <4	120	6	5	NS
				S/D >4	26	4	15	

SGA, Small for gestational age; *AGA*, average for gestational age; *SHR*, selected high-risk pregnancies; *Fet. Ao.*, fetal aorta; *EDV*, End-diastolic velocity present; *AEDV*, absent end-diastolic velocity; *Umb. a*, umbilical artery; *S/D*, systolic/diastolic ratio; *S/D N*, systolic/diastolic ratio normal; *S/D Abn.*, systolic/diastolic ratio abnormal.

cerebral blood flow velocity before 28 weeks. Indices of blood flow velocity remain unchanged during the last half of pregnancy in the uteroplacental circulation and the fetal aorta. However, in the umbilical artery and cerebral circulation, increasing end-diastolic blood flow velocity with decreasing indices to term requires gestational age–adjusted reference data for the interpretation of individual observations. No consensus has yet been achieved in regard to the criteria for abnormality for these measures. The common criteria used in current studies are absent end-diastolic velocity, indices of blood flow velocity outside the 95% percentile, or >2 SD beyond the mean for the gestational age.

It has been proposed that abnormal uteroplacental waveforms may reflect the raised vascular resistance resulting from inadequate physiologic change in the spiral arteries, which is characteristic of hypertension in pregnancy.[89] Some evidence has been presented to support an association between abnormal uteroplacental waveforms and pregnancy-induced hypertension in selected pregnancies at risk. However, a matched cohort study demonstrated that patients with or without hypertension may have abnormal uteroplacental waveforms. These authors recommended that "greater understanding of the determinants of uteroplacental waveforms is required before this technique is used to critically effect management."[92]

There is evidence to indicate that insufficient maternal or fetal circulation may be an important factor in impaired fetal growth. Alterations of uteroplacental blood flow in association with growth retardation have been indirectly measured.[143] Villous perfusion has been demonstrated to be significantly reduced in idiopathic growth retardation.[144] Animal research has suggested that increased placental resistance is associated with reduced umbilical blood flow and impaired fetal growth.[145]

Abnormal uteroplacental waveforms have been observed in association with growth retardation in selected population studies. However, the association has not been consistent, and the sensitivity of this finding for growth retardation has been modest. There is little evidence that this can be used as a screening test in the general obstetric population.

Initial studies of the association of fetal aortic or umbilical artery blood flow velocity with growth retardation were encouraging. However, subsequent studies have demonstrated sensitivities in the order of 50%, with a large number of false-positive results. Two studies that examined the value of these observations as a routine screening test in a general obstetric population with a known prevalence of growth retardation have failed to demonstrate a predictive role for abnormal umbilical artery blood flow velocity for growth retardation. Studies of fetal hypoxia in the animal model[146, 147] have demonstrated a "brain-sparing" effect with increased cerebral blood flow velocity in the growth-retarded fetus. However, the findings in regard to cerebral blood flow velocity in growth retardation have not been consistent.

Antepartum alterations of fetal blood flow velocity in the uncomplicated pregnancy may be due to the recently demonstrated relationships with fetal blood gases. For example, it is proposed that the reduced blood flow per kilogram of body weight in the fetal aorta near term may reflect the redistribution of cardiac output in response to a relative degree of hypoxemia.[113] Similarly, it is proposed that the increased cerebral

blood flow velocity and decreased pulsatility index in the cerebral circulation are a response to the relative decrease of fetal oxygen tension.[115]

A test to predict biochemically determined fetal asphyxia would be of great value. Concurrent measures of antepartum fetal blood gas and acid-base status and fetal blood flow velocity in complicated pregnancies have been limited. In these circumstances, although the degree of abnormality of blood flow velocity parallels the severity of fetal blood gas compromise, random variations are observed.[114] Similarly, the data to date do not indicate that antepartum fetal blood flow velocity is a good predictor of intrapartum fetal asphyxia as expressed by umbilical artery blood gas measurement at delivery.

Using fetal blood flow velocity for predicting clinical fetal distress during labor has been proposed because there is evidence of an association between abnormal fetal blood flow velocity and clinical fetal distress in selected populations. However, the sensitivities are moderate and the positive predictive values are low. The examination of these measurements as a screening test in the general population suggests that maternal and fetal blood flow velocity before labor or in early labor has limited value as a screening measure for fetal distress.

One randomized clinical trial of the value of umbilical artery blood flow velocity as a component of fetal assessment has been reported.[148] The authors concluded that the availability of these data led to better decision-making with a lower incidence of clinical fetal destress and emergency cesarean section during labor. Normal umbilical artery blood flow velocity resulted in longer periods of nonintervention with less infant morbidity.

The importance of maternal and fetal blood flow to fetal growth and development is widely acknowledged. The opportunity to assess maternal and fetal blood flow and blood flow velocity with Doppler untrasonographic technology has been a significant development. Continuing research to refine these techniques and improve our understanding should remain a high priority.

However, no evidence has emerged during the last 5 years to support the routine use of maternal or fetal blood flow velocity determinations as a diagnostic test in the general obstetric population. The recommendation of the 1986 consensus conference is still valid. The use of Doppler assessment in pregnancy should be limited to the investigational setting.

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Ultrasonography in the diagnosis of gynecologic disorders

J. Robert Willson, MD
Albuquerque, New Mexico

Ultrasonographic examination is being used with progressively greater frequency as an aid in diagnosis of gynecologic disorders but too often without consideration of whether information other than that obtained from clinical examination is needed and whether ultrasonography can supply it. The size, number, and position of pelvic masses and, in some instances, the type of tumor can be identified, but it is not often necessary when operation is clearly indicated. Ultrasonographic screening for diseases of the reproductive organs has been proposed, but its use for this purpose is limited and it is not cost-effective. It should be used as an adjunct to clinical diagnosis, not as a primary diagnostic procedure. (AM J OBSTET GYNECOL 1991;164:1064-71.)

Key words: Ultrasonographic diagnosis, gynecology, adnexal masses, ovarian cancer screening

The role of ultrasonographic examination in obstetrics is well defined, but its place in gynecology is less clear. The images produced by early equipment provided less information concerning the pelvic structures and their disorders than they did for the pregnant uterus and the fetus. Technologic improvements and increased experience in interpreting the images have made the evaluation of gynecologic disorders considerably more accurate than during early studies. Transvaginal ultrasonography provides clear images of the cervix, the uterus, the adnexa, and the cul-de-sac. A major disadvantage is that the effective focal zone of the transducer is short; masses that are beyond the range of the probe will not be clearly defined or will even be missed completely. In spite of the disadvantages transvaginal ultrasonography is clearly superior to transabdominal ultrasonography for evaluating the reproductive organs, and it can provide a visual image of the structures as they are being felt.

Early pregnancy and its complications

Since the advantages of ultrasonographic examination in the diagnosis of ectopic pregnancy, in determination of gestational age when dates are uncertain, in determination of the status of the embryo, and in evaluation of other complications of pregnancy are well known, they will not be discussed.[1, 2]

Gynecology

As image resolution has improved and with the introduction of transvaginal ultrasonography, ultrasonography is being used with greater frequency in nonpregnant women but too often without adequate consideration of whether additional information is needed, whether ultrasonographic examination can supply it, and whether it is cost-effective.

Pelvic masses. The experienced ultrasonographer can determine position, size, and gross morphology of adnexal masses in almost every instance, but it is more difficult to determine exact tumor type. Single or multiple clear cysts in an only slightly enlarged ovary are probably physiologic and not responsible for symptoms. Larger unilocular ones may be cystadenomas. Multiple small, bilateral clear cysts are characteristic of polycystic ovarian syndrome, but history and the hormone profile are better indicators of this disorder than is ultrasonography. Multiloculated cystadenomas, benign teratomas, endometriomas, and corpora lutea usually have characteristic appearances.[3]

It is possible to suspect malignancy on the basis of the ultrasonographic image,[4] but a definitive diagnosis cannot always be made. Benacerraf et al.[5] reported a 73% positive predictive value for malignancy in adnexal masses and a 91% negative predictive value for excluding malignancy. Benign tumors usually have sharp, well-defined margins and are more likely to be anechoic. Indistinct borders and the presence of a solid echo pattern suggest malignancy, and as echogenicity increases, so does the possibility of malignancy. Although ascites is usually present when a malignant tumor involves the peritoneum, there may be only a minimal amount of cul-de-sac fluid, or none at all, when the cancer is small and confined to the ovary or when there is only slight peritoneal involvement.

Although ovarian neoplasms and adnexal tumors may have typical ultrasonographic appearances, preoperative scanning of adnexal enlargements adds little but expense for the patient when there is a clear indication for operation. Too often, ultrasonographic ex-

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Reprint requests: J. Robert Willson, MD, University of New Mexico Hospital, Department of Obstetrics and Gynecology, 3211 Lomas Blvd. NE, Albuquerque, NM 87131.
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amination is ordered, simply because he or she palpates a pelvic mass. There is rarely a need for diagnostic imaging of any sort in women with obvious uterine leiomyomas and in those with unilateral adnexal masses ≥8 to 10 cm in diameter. These women can be operated on without further study.

Since one can usually suspect malignancy and anticipate the need for an extended operation by clinical evaluation alone, ultrasonographic examination should be an adjunctive rather than a primary diagnostic procedure in the study of adnexal masses. Color flow imaging may increase the accuracy when used for the diagnosis of cancers.[6]

Abnormal bleeding. A common cause of hypermenorrhea is the presence of submucous leiomyomas, which may not enlarge the uterus significantly and which may neither be felt with a curette nor be seen through a hysteroscope. Another cause is intramural myomas, which enlarge and distort the endometrial cavity. If the presence of myomas, their number, location, and size can be determined by ultrasonographic examination, an appropriate operative plan can be developed if myomectomy is being considered. Otherwise it is of limited value in these conditions; most therapeutic decisions can be made without it. Of course, ultrasonographic examination cannot replace biopsy to rule out malignancy as a cause of abnormal bleeding.

Pelvic pain. Ultrasonographic examination may indicate the cause of chronic pelvic pain, but in most instances the examination reveals no abnormalities. This is not surprising because of the many causes of chronic pelvic pain in which there is no anatomic change in the pelvic organs. Adhesions, which can be a factor in pain, may not be visible unless they distort the pelvic structures, the lesions of peritoneal endometriosis may be too small to be identified, and adenomyomas may not be detected. The fact that the examination reveals no source of the pain should not be completely reassuring.

Uterine cancer. Extension of cervical cancer into the parametrium and the depth of myometrial invasion of endometrial cancer can be determined by transvaginal ultrasonography, but the identification of extension to adjacent organs, lymph node involvement, and distant metastases is less accurate. Although thickness of the endometrium can be measured ultrasonographically and irregularities, fluid collections within the cavity, and endometrial polyps can be seen, biopsy is still essential in women with irregular bleeding, particularly those who are postmenopausal. A histologic diagnosis cannot be established, but ultrasonographically directed needle biopsy is a feasible procedure.

Adnexal infections. Ultrasonographic examination may be helpful in evaluating women thought to have acute salpingitis, particularly when there is a possibility that appendicitis may be causing the symptoms.[7, 8] It also may be helpful in diagnosing hydrosalpinx, pyosalpinx, and tuboovarian abscess and in monitoring response to antibiotic therapy. Pyosalpinges and tuboovarian abscesses can be drained and irrigated by ultrasonographically directed needle insertion.[9]

Reproductive endocrinology–infertility. In addition to follicle tracking in preparation for artificial insemination, follicle monitoring during ovulation induction, and ova retrieval, an infrequent use of ultrasonographic examination is to detect the occasional patient who is infertile because of the luteinized unruptured follicle syndrome. The hormone profile may be similar to that during normal ovulatory cycles, but the most informative noninvasive method of detecting failure to ovulate is serial ultrasonographic studies.

Postoperative complications. Ultrasonographic examination may provide important information in women with postoperative complications. Hematomas, abscesses, and unusual amounts of fluid blood within the peritoneal cavity can be identified. Abdominal wound dehiscence can be detected, as can hydroureter and hydronephrosis after ureteral injury.

Congenital müllerian anomalies. Fusion anomalies in the müllerian system can be identified by ultrasonography. With partial distal congenital vaginal aplasia and an intact uterus, for example, the blood-distended uterus, fallopian tubes, and proximal vagina can be seen and the extent of the defect determined.[10] Such examinations permit the surgeon to plan an appropriate operation. There should be no need for ultrasonographic examination in patients with imperforate hymen; the symptoms and the clinical appearance are characteristic. Uterine malformations, both during and in the absence of pregnancy, can be identified.

Screening. Ultrasonography is helpful in identifying or eliminating some disorders of the reproductive organs, but its role in screening has not been determined. If it does have an important screening potential, the most fruitful area might be in the detection of early ovarian cancer. Since most ovarian malignancies develop during the perimenopausal and postmenopausal phases of life, since most are advanced when diagnosed, and since the incidence increases with age, these are the women who should benefit most from screening.

Campbell et al.[11] performed 14,594 annual ultrasonographic screens in 5479 self-referred, asymptomatic women >44 years old. They identified five primary ovarian cancers, two at first screening and three at second screening; four were stage IA and one stage IB. They also identified an additional four patients with metastatic ovarian cancers.

Other than accuracy, an important factor in arriving at decisions on routine screening for ovarian cancer is cost-effectiveness. In the study of Campbell et al. a positive result was obtained in 338 screens of 326 women (2.3%); nine of these had either early primary

or metastatic ovarian cancer. This amounts to one abnormality in 432 screens and one cancer in 1621 screens. At an average charge of only $150 per screen (and this may well be low) the cost of diagnosing one ovarian abnormality would have been $64,800 and one ovarian cancer $243,150. In 1987 there were 5,744 million women aged 50 to 54 with life expectancies of about 30 years in the United States. If only half were screened only 10 times, the total number of screens would be 28.7 million, the total cost $4.3 billion, and the cost per ovarian cancer about $2.7 million.

Palpable postmenopausal ovaries. It is much easier to identify normal ovaries during reproductive life by both physical and ultrasonographic examination than the tiny, atrophic structures seen well after the menopause. Identification of both normal and abnormal ovaries should be more accurate with transvaginal ultrasonography than transabdominal ultrasonography, but atropic changes and constriction in the introitus and vagina may preclude the use of the vaginal probe.

Rulin and Preston[12] retrospectively compared ultrasonographic and operative findings in 150 postmenopausal women admitted for treatment of adnexal masses. Malignancy was found in only one of 32 ovaries that measured <5 cm in diameter. There were three invasive ovarian cancers, two borderline tumors, and one nonovarian cancer in 55 ovaries measuring between 5 and 10 cm, and there were 35 ovarian and five nonovarian cancers in 63 masses >10 cm in diameter. Goldstein et al.[13] identified 42 unilateral, unilocular cystic structures <5 cm in diameter in asymptomatic postmenopausal women by ultrasonographic examination. All 28 of the patients who underwent operation had benign ovarian or adnexal tumors. They proposed that serial ultrasonographic follow-up may be all that is necessary for women with small unilateral adnexal cysts that have none of the characteristic signs of malignancy. These findings suggest that operation is not always necessary when small ovarian enlargements are felt in postmenopausal women and that with more experience and with improvements in ultrasonographic technology it may become possible to determine which postmenopausal women with enlarged ovaries can be followed up safely.

Comment

Ultrasonographic examination is an important adjunct to clinical evaluation of the reproductive organs, but it is overused. There is no question of its value in identifying abnormal early pregnancy, in reproductive endocrinology and infertility, and for detecting and localizing pelvic masses, but other than these its role in gynecology is as yet unclear. Goldstein[14] has proposed that transvaginal ultrasonography be a routine part of every gynecologic examination and even that it be performed at each prenatal visit during early pregnancy. Clearly small, and often inconsequential, pelvic masses that are overlooked on physical examination will be identified by ultrasonographic examination. Conversely, the identification of small myomas, ovarian enlargements, and physiologic cysts may lead to increased patient concern and even to operations that might well be unnecessary. The cost of routine ultrasonographic examination does not justify its use.

As terrible a disease as ovarian cancer is, it plays a relatively small role as a cause of death in women. In 1986, 11,761 women >35 years old died of ovarian cancer, but 40,350 women in the same age group died of lung cancer, 39,865 of breast cancer, and 28,172 of colon and rectal cancer. It seems more logical to improve the availability and quality of health counseling and of preventive and early diagnostic services for these lesions and for cardiovascular and other potentially lethal disorders than to initiate ultrasonographic screening for ovarian cancer or for any other disorder of the reproductive organs.

Most reports on the accuracy of ultrasonographic examination in evaluating gynecologic lesions have been by experienced ultrasonologists with a background in diagnostic imaging. Obstetrician-gynecologists have usually learned ultrasonography from postgraduate courses and by the experience of interpreting their own films. It seems quite likely that the diagnoses made by the latter are less accurate than those made by diagnostic imaging professionals. Failure to diagnose obscure abnormalities or misinterpretation of the image opens the risk of legal action.[15] Ideally the acuity of obstetrician-gynecologists should be greater than that of ultrasonographers; the former have the advantage of having obtained a history and having performed pelvic examination whereas ultrasonographers study only the images. Diagnostic accuracy will undoubtedly improve in the future; ultrasonographic training in obstetric patients is now included in most residencies, but many lack comparable experience in imaging of gynecologic disorders. When expertise in this important adjunctive examination is expanded and when it is taught in departments of obstetrics-gynecology by obstetrician-gynecologists, most residents will become as competent in gynecologic ultrasonographic diagnosis as they now are in obstetrics.

Before the advent of ultrasonographic examination reasonably accurate diagnoses of early pregnancy and disorders of the reproductive organs were made by history and physical examination. Now one of the first thoughts of residents and many practicing obstetrician-gynecologists is to order ultrasonographic examination whenever pregnancy or an abnormality of any sort is suspected. Most such examinations could be avoided if physicians took the time necessary to obtain as much

historic information as possible, to examine patients skillfully, and to make diagnoses and establish treatment plans as though there were no backup alternative evaluative method. O'Brien et al.[16] found the preoperative clinical diagnosis to have been accurate in 67% of 52 women, whereas the ultrasonographic diagnosis was correct in only 42%. The diagnostic deficiencies of our residents stem from our not requiring that they continually improve their clinical diagnostic skills and from our permitting them to order diagnostic imaging and laboratory studies indiscriminately without considering whether they are necessary and how much additional information they will provide. Limiting the use of diagnostic imaging adds little risk for most patients and certainly reduces cost.

At the moment we would do well to accept the advice of two experienced ultrasonographers, Athey and Hadlock[17]: "Sonography of the female pelvis is most useful as an adjunct to the clinician's physical examination and should not be expected to provide histologic diagnoses. Once this is accepted by both sonologist and referring physician the sonologist can give a broad differential diagnosis without embarrassment and the referring physician will learn to appreciate the information gained and to interpret it in the light of the patient's clinical and physical findings." As both technology and experience in interpreting the films improve, ultrasonographic diagnoses will become more accurate and we will be able to identify lesions that are now overlooked. Even after this occurs, it is important that we insist that our residents develop and continually improve clinical diagnostic skills and learn to use ultrasonography as a secondary rather than a primary diagnostic tool.

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Discussion

Secretary James R. Scott. By way of explanation, about 2 years ago the American Gynecological and Obstetrical Society had a retreat—the Council had a retreat and decided that we should take a role in deciding important issues in our specialty, and particularly areas such as new procedures and new technology, areas that are changing rapidly and may be confusing to all of us and particularly to physicians in private practice.

I think I really believe that one of my rules that applies to us in medicine is that nothing really works as well for you and me as it says in the literature and that there are many reasons for that. There really are.

As you know, there's almost an inherent bias in publishing. We all get enthusiastic about new things, and we want them to be positive. If you think about it, very few negative studies are published, or if it doesn't work for somebody else, he or she is really not going to publish it; retractions are rarely published.

That, along with sort of an entrepreneurial approach to medicine, with marketing, promotion, and so on, it's very easy to get enthusiastic about new techniques that may or may not be proved.

We wanted to set up a system for this program whereby we would take one topic that's important to us in our specialty and look at it in a more objective way or critique it; we're going to try to do this every year.

This year it was decided that we would take ultrasonography; specifically, we wanted people who are not ultrasonographers to look at the area. We picked what we hope, what we think, is a distinguished group that were given 1 year to look at this topic and give us a presentation that is not a position statement but is being published along with the rest of the articles from this meeting.

DR. PITKIN. The important part of this panel is the time for discussion and questions and comments from the floor, and it is now open for that purpose.

DR. FRANK CHERVENAK, New York, New York. Dr. Pitkin, that was an excellent conservative review of the value of ultrasonography in the diagnosis and management of fetal anomalies.

There is one point I hope we could clarify. We agree that ultrasonography is very valuable in the detection of many fetal anomalies; we also agree that this detection of anomalies can have very serious consequences in management options for the pregnant woman. In particular, in the absence of early detection of anomalies, she would not have the option of termination of pregnancy if those anomalies are diagnosed later in pregnancy or after birth.

In addition, for many other anomalies there are admittedly a few cases where fetal therapy and aggressive management, such as delivery in a tertiary center, can help.

It's also a fact that the ethical principle of respect for autonomy is a central principle in clinical obstetrics. Indeed, some authors would argue that it's the single most important ethical principle in the practice of obstetrics today. This principle has its most important application in clinical practice where medicine is a bit uncertain about the benefits and harms of diagnostic or therapeutic interventions.

The question I want to pose to you is, do you have any argument that would defeat an autonomy-based obligation of our profession to offer ultrasonographic examinations to the low-risk patient in clinical settings where quality—and I emphasize quality—ultrasonography is available? Offering such an option would fulfill the obligation of our profession to respect that autonomy. At the same time it would not force premature closure on this important debate and give us time to do the cost-benefit analysis and randomized studies that hopefully would allow us to come to a more definitive recommendation for or against routine ultrasonography.

DR. PITKIN. Those of you who have read Dr. Chervenak's recent article (Chervenak FA, McCullough LB, Chervenak JL. Prenatal informed consent for sonogram: An indication for obstetric ultrasonography. AM J OBSTET GYNECOL 1989;161:857-60), arguing on the basis of an ethical analysis for obstetric ultrasonography on patient request, will better understand the nature of his question.

Although perhaps everything is ethical in the final analysis, I would not cast this in the traditional ethical mode. Rather, I view it as a professional obligation and I personally have a difficult time whereby I am entirely unclear as to the proper action, saying to the patient, "You decide, because the issues are too complex for me to reach a conclusion."

I would not take issue with offering that service, but I would argue with its becoming the standard of practice before the important issue of cost-effectiveness has been dealt with.

DR. JAMES AIMAN, Milwaukee, Wisconsin. My question concerns screening ultrasonography. Accepting your figure of a 2% to 3% prevalence of anomalies, would you not expect a specificity of ultrasonography above 95%? Would you not achieve that specificity if you did nothing or merely palpated the abdomen, given the prevalence of anomalies?

DR. PITKIN. You are absolutely correct; specificity is 100 minus the prevalence.

DR. DAVID GRIMES, Los Angeles, California. I'll provide an epidemiologic footnote to Dr. Aiman's comment. There's a useful rule of thumb when one is interpreting the validity of a diagnostic test and that is that the sensitivity and specificity of a good test should add to at least 1.5 or 150%. The sensitivity and specificity of a very good test should add to at least 1.8; what we've seen in the past 45 minutes is that by that criterion, ultrasonography falls short for many indications.

DR. GEORGE D. WILBANKS, Chicago, Illinois. I certainly want to applaud Dr. Willson's comments about teaching the pelvic examination. I'm in the process of writing an article on the lost art of the pelvic examination, but I would not like to throw out the use of ultrasonography in screening for ovarian cancer and perhaps other diseases. I think the costs looked very big in this study, but as we are seeing in our residency program (ultrasound experience with the assisted reproduction program in endocrinology), residents are going to be very good with vaginal ultrasonography. It could be done very cheaply in the office setting by the obstetrician-gynecologist.

We also need to add the possibility of looking at endometrial cancer and ovarian cancer with the same screen. If we eliminate some of these sonographic examinations by the serum markers that we heard about today, such as CA 125 and interleukin 6, the costs should be more reasonable, but we should not eliminate ultrasonographic screening until we have more data.

DR. A. BRIAN LITTLE, Montreal, Quebec, Canada. I have two questions for Dr. Willson. The first one has to do with the pelvic examination. It seems to me that when chest roentgenography came along we suddenly found that the chest was quite different from what we anticipated with examination by stethoscope. I think it's particularly difficult in this day and age to teach people how to do pelvic examinations when so many people are immediately referring patients to ultrasonography without an examination.

So often we see women who have had ultrasonography and then are referred to the gynecologist, rather than the reverse. I have taken the position that it's probably not possible to teach anyone to do what some of us believe an examination should be after 20 or 30 years of experience without ultrasonography.

The second issue is that you didn't mention anything about vascularity, which ultrasonography can identify in pelvic masses. Masses can now be examined with Doppler techniques and an estimate of the degree of vascularity can suggest the presence of malignancy.

DR. WILLSON. I did mention color flow, which is vas-

cularity. As far as pelvic examination is concerned, I don't think we can throw out pelvic examination nor do I think we can substitute ultrasonography for pelvic examination.

Goldstein, for example, has suggested that every pelvic examination done in our offices should include an ultrasonographic examination and also that it should be done at every prenatal visit during early pregnancy. This, I think, is carrying it a bit too far.

I'm fully aware of the inaccuracies of pelvic examination; we don't find very small things, but the vast majority of small things that we miss are inconsequential. I've been working at pelvic examinations for several years, and I am no more completely accurate than anyone else. However, I don't think I need to substitute ultrasonography for my pelvic examinations, and I think residents can learn pelvic examination if we insist on it.

Dr. Timothy Johnson, Baltimore, Maryland. Dr. Pitkin, I think you passed over an issue that for some of us remains problematic—the political issue of ultrasonography and "turf." I see more and more other specialties getting involved with it—I recently heard of am emergency room doctor who wanted to get an ultrasound machine for the emergency room so he could do a vaginal probe ultrasonographic examination to rule out ectopic pregnancy before he sent the patient home. I suspect that, as Dr. Little just mentioned, more and more women are referred from physicians who use ultrasonography to monitor their patients, almost as a surrogate pelvic examination.

I guess it would be very useful for me if the distinguished members of the panel could tell me what their feeling is about the state of the art in 1990 with respect to our responsibility as obstetricians-gynecologists in performing and controlling the imaging modalities.

Dr. Pitkin. I'll respond first. I agree that the gynecologist's role in ultrasonography is a key one, but I do not feel that any medical specialty has a right to control it. Rather, I think it ought to be available for use by those who are experienced with it. The ultimate goal should be to help the patient rather than to exert political control.

Dr. Low. Our responsibility is to answer the questions that Dr. Pitkin posed. Is it safe? Does it work and is it cost-effective? If we do that, professionally speaking, we're creating the answers, which will ultimately lead to the practice.

As Dr. Pitkin pointed out, there is a tendency in health care for many things to be seriously misused for a while until the real truth comes home.

Dr. Pitkin. Dr. Willson, do you have a feeling about the turf issues?

Dr. Willson. I think the same thing ought to happen to gynecologic ultrasonography that happened to obstetric ultrasonography. It started with the ultrasonographers and gradually has become a service offered in departments of obstetrics-gynecology. If we do the same thing with gynecologic ultrasonography, then I think all concerned will end up with a better result—the patients, the residents who are learning, and ourselves.

Dr. Steven G. Gabbe, Columbus, Ohio. I want to congratulate the members of the panel. I think it's refreshing for those of us who spend a lot of time in darkened rooms looking at a lighted screen to evaluate these data objectively. I think the panel was able to do that.

A recent article from Finland (Saari-Kemppainen A, Karjalainen O, Ylostalo P, et al. Ultrasound screening and perinatal mortality: Controlled trial of systematic one-stage screening in pregnancy. Lancet 1990;1:387) showed, in a study involving >9000 pregnant women, an improvement in perinatal mortality, primarily because of the detection of malformations in women who did have a routine ultrasonographic examination. I would like to ask Dr. Pitkin to address a few of the other advantages espoused for routine ultrasonography; that would include improved dating of pregnancies. This would allow a more accurate interpretation of α-fetoprotein levels, which are obtained in most of our practices, earlier detection of twins, avoidance of hemorrhage from undetected or unrecognized placenta previa, and finally the psychologic benefits to the patient and her partner.

Do you have comments on these?

Dr. Pitkin. Although my topic was limited to screening for malformation, you've raised the broader matter of perinatal mortality in general. We must remember that old saw, "A difference, to be a difference, must make a difference." If ultrasonography detects lethal malformations early enough for these fetuses to be aborted, perinatal mortality rates should improve. I'm not familiar with the randomized trial that you mentioned, but the ones I do know have not demonstrated any clear improvement in perinatal outcome. Yes, twins can be diagnosed earlier; yes, gestational age can be fixed more accurately; yes, the placenta can be localized. But does it make any difference in outcome statistics? If not, I would argue that routine usage is difficult to support.

Dr. Robert Sokol, Detroit, Michigan. I would like to attempt to provide what might, perhaps, be considered a little balance to this discussion, at least from the perspective of a perinatologist.

Beginning with Doppler flow, I think it's fair to state that a clinical role, if any, for developing this technique is under intensive study. However, I believe that there is reasonable consensus that at this time it remains a research tool.

With regard to the views presented at this meeting concerning other ultrasonographic modalities, I think it's fair to state that they are far to the "right," in terms of the views of most maternal-fetal medicine subspecialists and prenatal geneticists, not to mention in terms of a reasonable interpretation of available studies and clinical experience.

Consider, for example, the statistical argument presented concerning predictability. Because congenital anomalies statistically are rare events, achieving im-

pressive sensitivity and specificity is difficult; indeed, guessing in the proportion of the prior probability typically will produce the best overall correct classification rate. For example, if an anomaly occurs at a rate of 1%, if you are faced with 100 pregnancies and you make 99 "no" guesses and 1 "yes" guess, you will be right 99% of the time. The problem is that your chance of being correct for a specific patient is only 1%, and missing an anomaly that could be detected easily during ultrasonographic examination is costly and clinically unacceptable. Sensitivity and specificity are not really the issue here.

Furthermore, our group has reported that visualization of fetal organs in the second trimester was distinctly limited in the mid-1980s, the period from which most of the studies in this review were derived. We will shortly report a very substantial improvement in ultrasonographic visualization of fetal organs, based, in part, on improved hardware.[2]

I suppose you could just forget trying to detect anomalies, but, in fact, being able to detect 20% to 30% of them is, for the individual clinician and patient, most assuredly of value. And I think our detection rate has improved and will continue to improve.

A brief final note as a follow-up to Dr. Little's comment on the gynecologic examination: Most of us who trained many years ago believe that we do very good pelvic examinations, but I believe that we could tell more by seeing than by feeling alone. While the technology is still under development, an ultrasonographic transducer that fits on the fingertip under the glove will soon be available. We will be able to visualize what we are feeling. Don't you think we'll be able to perform a much better pelvic examination? Time and technology march on.

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DR. LITTLE. I have one more comment because I have to put money into ultrasonography like many other chairmen here. I'm enthusiastic but conservatively reluctant at the same time.

I wonder whether you're examining the wrong end point. In other words, your results are evaluated over a base number of 10,000 pregnancies. One of the biggest epidemiologic problems in obstetrics is to find an end point that will show a difference without considering large numbers. This is related to Dr. Sokol's comment that when you find a number of women who have these particular problems identified by early ultrasonography, a tremendous change takes place in the management of that pregnancy. When you find an abnormality, although not significant in your study, it is a very significant observation for that small number of patients.

I know that the overall cost makes a difference, but a positive result makes a big difference to that number. When you start thinking about this in terms of the rest of medicine, we're always trying to justify costs with the thousands of women or millions of women who have babies, other than the few who have an abnormal fetus. There's no question that the group with these abnormalities have a significant change in the way their pregnancies are managed and in the potential outcome after such a diagnosis. This could justify at least some extra cost.

DR. ALLEN P. KILLAM, Durham, North Carolina. I think the answer is that it is important to say that to make a difference, it has to make a difference. But I think if you're comparing standard obstetric care with any new interventions such as electronic fetal monitoring, we find that the risk to the patient is very low, and on a statistical basis to detect a real difference when the risk is very low requires huge numbers.

Yet we as practitioners are faced with a patient who says, "Shouldn't I have an ultrasound if I want it?" and you say, as an epidemiologist, "Well, we can't prove a cost-effective ratio." Yet if that patient wanted an ultrasonographic examination and you didn't do it and she were the very unusual patient to have a problem, then the hassle to us of not having offered it, even though we can't prove that statistically it's important, is so great that I think we end up, in practice, doing electronic fetal monitoring and using ultrasonography as a practical approach to avoid the very unusual case where it makes a difference, although it doesn't show up as statistically significant in our evaluations.

DR. PITKIN. Certainly, these kinds of considerations have influenced practice in a variety of ways over the years.

DR. DAVID COTTON, Houston, Texas. I saw you had a tremendous spread in the amount that physicians charge for an ultrasonographic examination. I would be interested in a comparison with what obstetricians charge for an initial physical examination and prenatal blood panel during pregnancy and what they charge for ultrasonography. In many states I would estimate the cost of a prenatal panel to be $100, and $50 to $75 for that initial physical examination.

My question is, would you feel more comfortable if the charges for ultrasonography were standardized in each community and if much lower rates were used? If that standardization occurred, would the cost-benefit analysis become favorable in your mind?

DR. PITKIN. Certainly, if you lower the cost and hold the benefit constant, then the cost-benefit ratio must decrease.

DR. COTTON. I guess what I am really asking is whether a physical examination of the fetus with ul-

trasonography is more cost-effective than a physical examination of the mother by her obstetrician?

DR. PITKIN. I don't know. Dr. Gant?

DR. NORMAN F. GANT, Dallas, Texas. This sort of reasoning just drives me insane. Here we've got an unbelievably technically difficult procedure to do, and we're talking about screening all the women in the United States with it. Even if it were the best screening technique today, which it isn't—and we've had evidence that it isn't—but even if it were, we couldn't implement it for another 20 years until we could train the people to do it and interpret it.

I think this is a form of mental gymnastics. What we're talking about here is whether someone could afford it. Let's lower it to $25. Well, if you lowered it to $25, you would still get the same result. You would have excellent people doing it who are publishing the articles, and then you would have the rank and file like me, who are frightened to turn on the machine because they're afraid they'll break it.

We have a technical skill here that in wonderful hands is not quite good enough, and that's what it is.

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Clinical Opinion

Sacrospinous fixation—Should this be performed at the time of vaginal hysterectomy?

Stephen H. Cruikshank, MD
Minneapolis, Minnesota

A sensible individualized approach should be applied to every patient undergoing transvaginal surgery for benign disease. This approach should attempt to correct every defect present in the pelvic supports. Uterovaginal prolapse is the result rather than the cause of genital prolapse. Not every vaginal hysterectomy should be treated like a cystocele-rectocele repair. Instead, every defect of the endopelvic fascial support should be evaluated in a patient both before and during surgery. As a result of these evaluations, more than just a hysterectomy and an anterior and posterior colporrhaphy may be performed. In a case in which a patient is found to have more than one defect at the time of examination, sacrospinous fixation of the vaginal apex at the time of transvaginal hysterectomy may be indicated. In the office, the patient can be examined in the supine and standing positions, both with and without Valsalva's maneuver, to determine if moderate to severe uterovaginal prolapse exists. Sacrospinous fixation should be performed in those cases as an adjunct to other steps taken to prevent posthysterectomy prolapse. (AM J OBSTET GYNECOL 1991;164:1072-6.)

Key words: Prophylaxis, uterovaginal prolapse, sacrospinous fixation

Vaginal vault prolapse is an infrequent but tragic complication that may occur after hysterectomy. Its incidence ranges from 0.2% to 43%. Both American and European literature are filled with descriptions of methods of repairing vault prolapse. Primarily as a result of the work of Nichols and Randall[1, 2] in the United States, the use of sacrospinous fixation to correct this condition has had a resurgence in our country. Others have stated that sacrospinous fixation should be used only for therapy of posthysterectomy vault prolapse.

The word *prophylactic* as it pertains to the use of sacrospinous fixation at the time of hysterectomy has been misunderstood by many. According to *Webster's Dictionary, prophylactic* means to prevent or to ward off. This is indeed what we would like to do when it comes to posthysterectomy prolapse—prevent or ward off this complication. The word *prophylactic* does not mean *routine*. If we examine a patient who has a defect of the uterine ligaments, as well as other defects in the vaginal supportive structures, we should think about adding an additional measure to prevent posthysterectomy prolapse.

From the Department of Obstetrics and Gynecology, University of Minnesota, and Hennepin County Medical Center.
Reprint requests: Stephen H. Cruikshank, MD, Department of Obstetrics and Gynecology, Hennepin County Medical Center, 701 Park Ave. South, Minneapolis, MN 55415.
6/1/24738

This article recommends the use of sacrospinous fixation at the time of hysterectomy as an adjunctive measure in those patients who are at risk for vault prolapse. A clinical definition of patients at risk for future vault prolapse is provided. Also described is a method of attaching the uterosacral cardinal ligaments to the vaginal membrane to help prevent vault prolapse in patients who undergo hysterectomy. In addition, material relevant to sacrospinous fixation at the time of hysterectomy is described with illustrations.

It is important that physicians who treat patients with pelvic floor relaxation consider each aspect of the supportive structures of the pelvic floor. I think that it is of value to keep this in mind when the patient is evaluated in the office. An excellent history and physical examination with the patient in erect and supine positions are of utmost importance. After the physician has determined whether the patient has genital relaxation, an attempt should be made to identify in which anterior-posterior area the defect occurs and then the repair can be planned. Uterine prolapse is not the cause of genital prolapse; it is the result. Are we really sure what causes anterior wall relaxation, posterior wall relaxation, or an enterocele that is the result of genital prolapse? I don't think so, but I think that we should do everything that is therapeutically available to prevent the hazards of not totally repairing all defects at the time of hysterectomy. All relaxations should not be treated as cystocele-rectocele.[3]

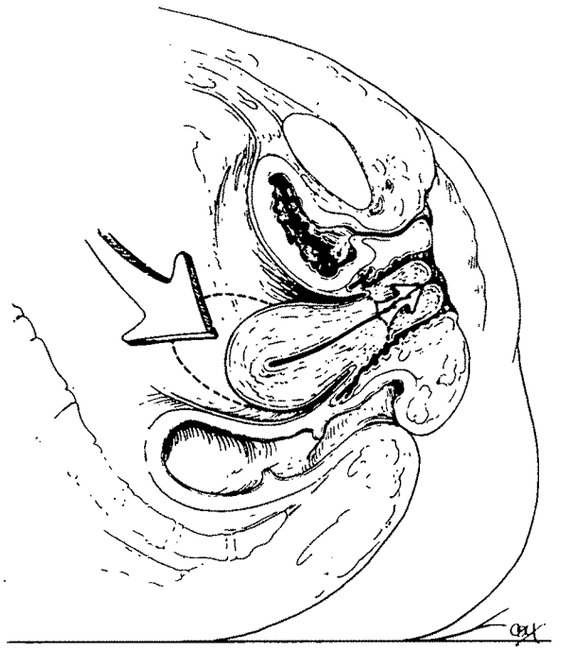


Fig. 1. Moderate uterovaginal prolapse. (From Cruikshank SH, Cox DW. Sacrospinous fixation at the time of transvaginal hysterectomy. AM J OBSTET GYNECOL 1990;162:1611-9.)

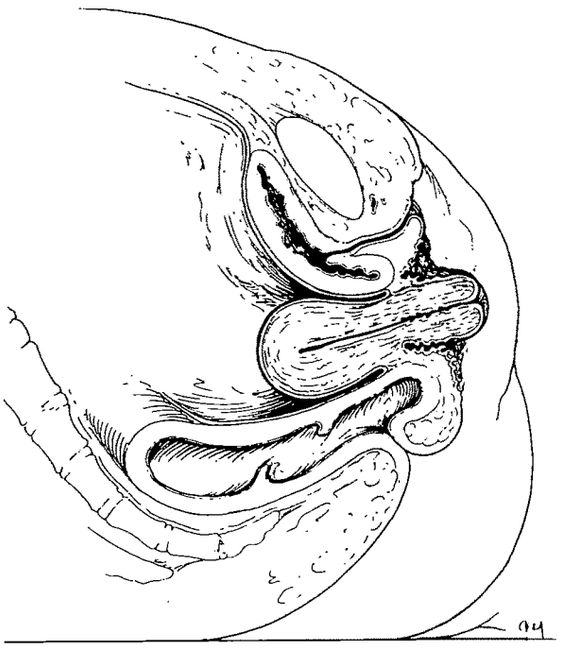


Fig. 2. Severe uterovaginal prolapse. (From Cruikshank SH, Cox DW. Sacrospinous fixation at the time of transvaginal hysterectomy. AM J OBSTET GYNECOL 1990;162:1611-9.)

Genital prolapse (uterine-vaginal prolapse, cystocele, rectocele, enterocele) represents a disorder of the pelvic support. We must determine whether the prolapse was caused by childbirth, pelvic trauma, or stretching or laceration of the connective tissue of the vagina or by the more common causes, postmenopausal atrophy and attenuation. Some authors think that genital prolapse may result from a neuropathy or a congenital defect of the elastic tissue of the pelvic supportive structures. Last but not least, genital prolapse can be caused or exacerbated by chronically increased abdominal pressure associated with obesity, masses, chronic respiratory diseases, or occupational hazards.

The symptoms of uterovaginal prolapse include bulging with sitting or walking, ineffective control of or difficulty with urination or defecation (these patients are predisposed to urinary tract infections), irregular spotting or discharge associated with ulcerations, interference with coitus, or increased pelvic pressure or backache. When we see patients with these problems in the office for the initial evaluation, it is important to determine how to treat these patients surgically. Our objective is to restore the abnormal state to its normal anatomic position, to restore function, and to perform the appropriate procedure that results in the least chance of recurrence of the problem. When more conservative treatments such as exercise, estrogen therapy, and the use of pessaries in certain patients fail, transvaginal[2, 3] or transabdominal[4] surgical treatment of genital prolapse is indicated. We must also look for other defects and repair them at the time of surgery. If patients present with moderate or severe uterovaginal prolapse or procidentia, a sacrospinous fixation can be added at the close of hysterectomy and any other plastic repairs that replace the vagina in its near-horizontal position above the levator ani.

We should also look for defects in the endopelvic fascia and the genital supportive structures of patients who meet the criteria for transvaginal hysterectomy and whose primary indication for hysterectomy is other than uterovaginal prolapse. If a patient presents with moderate or severe *uterovaginal* prolapse or symptomatic pelvic relaxation, she may fulfill the criteria for having adjunctive sacrospinous fixation. *Moderate uterovaginal prolapse* (not uterine, but uterovaginal) is defined as the presentation of the cervix past the midportion of the vagina or to the introitus as a result of Valsalva's maneuver (Fig. 1). *Severe uterovaginal prolapse* is defined as the presentation of the cervix past the introitus either with or without Valsalva's maneuver (Fig. 2). If the uterosacral-cardinal ligament complex is found to be totally lax (surgically weak) or nonexistent, as it is in many cases of procidentia, then these structures will be unavailable to shorten or attach for support. An attempt should be made, however, to attach these ligaments in addition to performing sacrospinous fixation.

Traditionally, sacrospinous fixation has been regarded as a therapeutic tool to be used only for the repair of vaginal vault prolapse. However, I believe that

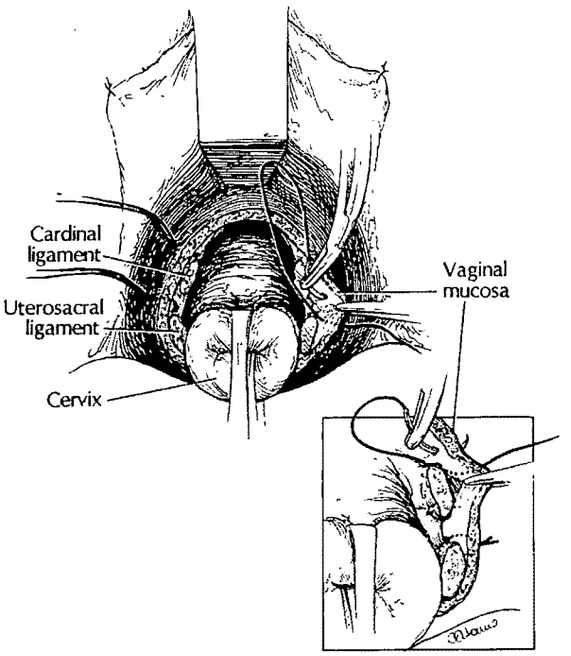


Fig. 3. Attaching uterosacral-cardinal ligaments to vaginal membrane. (From Cruikshank SH. Preventing posthysterectomy vaginal vault prolapse and enterocele during vaginal hysterectomy. AM J OBSTET GYNECOL 1987;156:1433-40.)

this procedure also may be used as a preventive measure in those patients who are at risk for posthysterectomy prolapse. Not every patient undergoing vaginal hysterectomy is a candidate for sacrospinous fixation. If a loss of the pelvic supportive structures (uterosacral-cardinal ligament complex) is noted at the time of hysterectomy, then an attempt to use their remnants should be made. However, sacrospinous fixation as an adjunct will prevent further vault prolapse. In *Vaginal Surgery,* 3rd edition, Nichols[1] states that in the absence of strong pelvic support a sacrospinous colpopexy is easy to perform after vaginal hysterectomy.

When a patient has been evaluated and has been found to have moderate to severe uterovaginal prolapse or procidentia, other defects in the vaginal wall can be identified. If necessary, anterior colporrhaphy, posterior colporrhaphy, and enterocele repair (or a procedure to prevent enterocele) can be performed. At the close of anterior colporrhaphy, the vaginal apex can be pulled toward the introitus with an Allis clamp. If the apex comes down to the introitus or past the introitus, the patient is probably at risk for future posthysterectomy vaginal prolapse. This patient should undergo sacrospinous fixation at that time, after which any other plastic repairs can be performed and the sacrospinous stitch can be tied to secure the apex against the sacrospinous ligament.

I like to attach the uterosacral and cardinal ligaments to the angles of the vaginal membrane in all patients undergoing transvaginal hysterectomy (Fig. 3).[5] This should be done as an initial step after the anterior and posterior colpotomy. If the structures are not too attenuated or lax and do not need shortening, this step can be undertaken immediately and will accomplish three goals: (1) It will help prevent posthysterectomy prolapse; (2) it will tie the angles of the vagina, which is a common site of postoperative bleeding; (3) it will prevent the physician from inadvertently cutting the sutures without having attached them to the vaginal membrane at the end of the operation. Many times these structures are too lax and need to be shortened and reattached after uterine extirpation. If the vaginal apex still appears at the introitus, then a sacrospinous fixation can be undertaken. The ligament fixation procedure is performed as follows: The posterior wall is opened to the apex. This is full-thickness vaginal membrane. The incision should be through the endopelvic fascia, which places the operator in the rectovaginal space. This space is dissected by the operator to the level of the ischial spines; however, the ischial spines and the coccygeus muscle cannot be identified at this time because the descending rectal septum must be perforated. At the time the descending rectal pillar is perforated, the pararectal space is opened (Fig. 4). The ischial spine and the coccygeus muscle–sacrospinous ligament complex are palpated and visually identified via additional blunt or sharp dissection. Number 2 Vicryl sutures are then placed by direct visualization through the ligament approximately 2 to 3 cm medial to the ischial spine. These sutures are held and left untied until any additional reconstructive procedures are completed. When the other procedures have been completed, the sacrospinous ligament fixation is carried out via both the safety stitches and pulley stitches (Fig. 5).

To date there is only one moderate-sized series that documents the usefulness of the sacrospinous fixation as an adjunct to transvaginal hysterectomy.[6] In that study transvaginal hysterectomy was accomplished in 135 consecutive patients. Forty-eight adjunctive sacrospinous fixations of the vagina were carried out in these same patients, who had presented with primary complaints plus additional uterovaginal–symptomatic outlet relaxation. There were no cases of injury to the rectum, nerves, or bladder in any of the 48 cases. Significant bleeding from the perirectal veins occurred in only two cases, but the bleeding stopped with pressure and no sequelae resulted.

In this same study the morbidity and complications were low. All patients received prophylactic cephalosporin antibiotics. Ninety-two percent of the 48 patients remained afebrile throughout the postoperative course. Of the four patients with febrile morbidity, three had urinary tract infection and one had no cause found. The average blood loss for all the procedures done was 290 ml; the average blood loss for sacrospi-

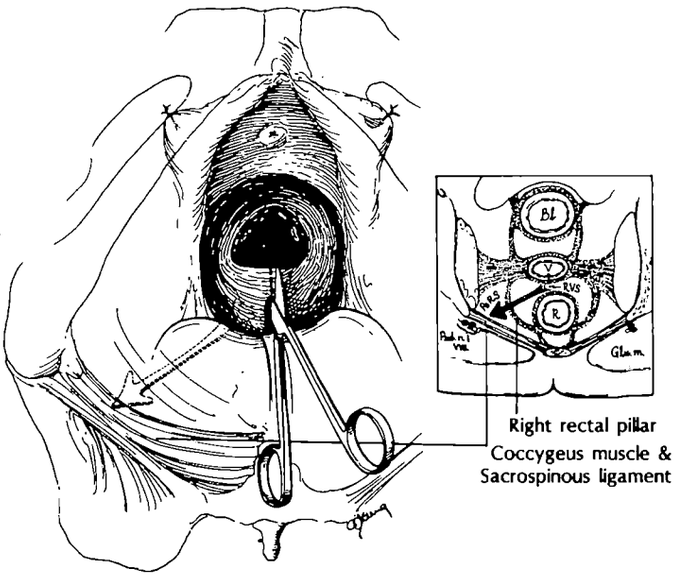


Fig. 4. Dissecting rectovaginal and pararectal spaces. (From Cruikshank SH, Cox DW. Sacrospinous fixation at the time of transvaginal hysterectomy. AM J OBSTET GYNECOL 1990;162: 1611-9.)

nous fixation was 100 ml. The average time for the entire operation, which was performed in a teaching setting, was 2 hours 20 minutes, and the average time for the sacrospinous fixation was 18.5 minutes. In this study some minor complications had resolved by the time the patient returned for the 6-week postoperative checkup. Four patients who underwent anterior colporrhaphy were unable to urinate before discharge from the hospital and were sent home with Foley catheters in place. All four patients were able to subsequently urinate. Two patients had transient stress incontinence from the sacrospinous procedure, but this complication also resolved in both cases. Twenty patients had right buttock pain that resulted from adequate dissection in the perirectal space and direct visualization of the sacrospinous ligament. No patients had resultant sciatica or neuropathies as a result of the procedure. All 20 of these patients had spontaneous resolution of buttock pain by the 6-week checkup. All patients underwent follow-up for 1 year 6 months to 3 years 8 months after surgery; posthysterectomy vault prolapse occurred in only one case. All patients were examined in both standing and supine positions at the 6-week postoperative visit and were asked to perform Valsalva's maneuver so that partial prolapses or missed enteroceles could be identified. The mean follow-up time of this study has now been more than 3 years.

In this same study 25 patients had anterior colporrhaphy to correct urinary stress incontinence, symptomatic anterior vaginal wall relaxation, or both. Eighteen patients had posterior colporrhaphy in addition to an anterior repair. Five patients had enterocele repair, and seven additional patients had posterior colporrhaphy only. Only one patient had a recurrent posthysterectomy vault prolapse.

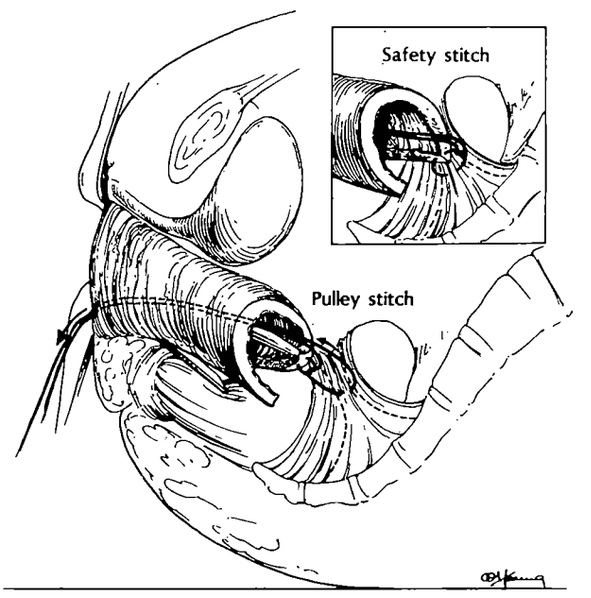


Fig. 5. Sacrospinous fixation demonstrating pulley and safety stitches. (From Cruikshank SH, Cox DW. Sacrospinous fixation at the time of transvaginal hysterectomy. AM J OBSTET GYNECOL 1990;162:1611-9.)

Comment

There have been many previously described methods that use the pelvic supportive structures (cardinal-uterosacral ligament complex) to help prevent posthysterectomy vault prolapse. These methods hold the

vagina in its horizontal position above the levator ani; however, if these supportive structures are not present, the vaginal apex is still at risk for prolapse. In this study all 48 patients had the apex drawn past the introitus after hysterectomy and plastic repair of the anterior wall. I feel that it is appropriate during transvaginal hysterectomy to support the vagina in its normal anatomic position. Moreover, if moderate or severe uterovaginal prolapse exists, either as a main indication or as a secondary diagnosis, it can be corrected with sacrospinous fixation to prevent future complications and additional procedures for the patient. Sacrospinous fixation is not to be performed universally at the time of hysterectomy. In the study cited, only 35% of the 135 patients who underwent vaginal hysterectomy fulfilled the prerequisites for sacrospinous fixation of the vaginal vault. I feel this is an appropriate adjunctive or prophylactic procedure if strict criteria are met: (1) total laxity of the uterosacral-cardinal ligament complex, (2) total procidentia at the time of hysterectomy, (3) moderate to severe laxity of the supportive structures and the ability to pull the vaginal apex to or past the introitus after hysterectomy and other plastic repairs have been performed.

Various principles of vaginal fixation are recognized today. I think that it is paramount that we note the laxity of any supporting structures and that we attempt to repair them at the initial operation, which affords the best chance of success. Transvaginal hysterectomy is a good method of preventing future vaginal prolapse. It seems feasible to add sacrospinous fixation to vaginal hysterectomy in patients who present with moderate to severe uterovaginal descensus and loss of pelvic support. Not every patient undergoing vaginal hysterectomy is a candidate for sacrospinous fixation. The procedure carries minimal morbidity, it supports the vaginal apex, and it provides pelvic surgeons with an additional adjunctive or prophylactic measure against posthysterectomy prolapse.

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Carrier screening for cystic fibrosis: Implications for obstetric and gynecologic practice

Sherman Elias, MD,[a] George J. Annas, JD, MPH,[b] and Joe Leigh Simpson, MD[a]
Memphis, Tennessee, and Boston, Massachusetts

Cystic fibrosis, which is one of the most common autosomal recessive disorders, has a carrier frequency of approximately 1 in 25 among whites in the United States. In this population approximately 75% of the mutations in patients with cystic fibrosis correspond to a 3 base pair deletion that results in the loss of a phenylalanine residue at amino acid position 508 (designated ΔF_{508}) from the coding region of the cystic fibrosis gene. Currently, only about half of the couples at risk can be identified as cystic fibrosis carriers. We support conclusions of the National Institutes of Health Workshop on Population Screening for the Cystic Fibrosis Gene, which state that carrier testing should be offered to all individuals or couples with a family history of cystic fibrosis. Good science and solid educational and counseling strategies must be in place before screening for cystic fibrosis is routinely offered to those with a negative family history. Pilot programs that investigate research questions in the delivery of population screening for cystic fibrosis carriers are urgently needed. (AM J OBSTET GYNECOL 1991;164:1077-83.)

Key words: Cystic fibrosis screening, ΔF_{508} mutation

Physicians and their patients face a continually increasing array of diagnostic tests that demand decisions regarding clinical use. Unfortunately, the decision to offer a new test has in the past sometimes been driven by the desire to avoid a potential lawsuit for not testing (defensive medicine). The medical profession is currently faced with another new screening test that has been heralded with banner headlines in the nation's newspapers—carrier screening for cystic fibrosis. Which position should obstetricians take regarding the use of this test in their practices, and what should they tell their patients about it? This article summarizes the current state-of-the-art in medical testing and suggests how the nature of the test and the applicability and reliability of its results lead to conclusions that support its limited use in current clinical practice.

Cystic fibrosis is the most common life-threatening autosomal recessive disease among North American whites of European ancestry. In this population the disease frequency is about 1 in 2500 live births, and the calculated carrier frequency is about 1 in 25 individuals.[1] Common clinical manifestations of cystic fibrosis include meconium ileus, chronic obstructive pulmonary disease that results in bronchiectasis and respiratory failure, pancreatic exocrine insufficiency with steatorrhea, liver cirrhosis, and failure to thrive.[1] The clinical course of cystic fibrosis is highly variable and ranges from death in the neonatal period as result of complications of meconium ileus to protracted survival into the fifth and sixth decade. Approximately half of patients with cystic fibrosis can be expected to live to age 26 or longer.[1]

Until the mid-1980s, families known to be at increased risk for cystic fibrosis could be offered only genetic counseling that used calculated risks that were based on mendelian inheritance. In recent years, however, a number of important advances have been made. First, microvillar-intestinal-enzyme levels have proved to be decreased in the amniotic fluid of women carrying fetuses affected with cystic fibrosis, although the sensitivity and specificity of this assay were less than optimal.[2] Later, use of polymorphic deoxyribonucleic acid (DNA) markers closely linked to the cystic fibrosis gene locus on chromosome 7 made prenatal diagnosis possible, with the use of either amniotic fluid cells or chorionic villi, with a very high degree of accuracy for most couples; however, DNA from the previously affected relative was necessary.[3, 4] For close relatives of cystic fibrosis patients, carrier testing by DNA linkage studies also could be provided with a high degree of accuracy.[5]

Within the past year, the cystic fibrosis gene has been cloned and sequenced.[6-8] The protein presumed to be encoded by the cystic fibrosis gene has been named the cystic fibrosis transmembrane conductance regulator and is believed to be a membrane-bound transport molecule. However, the manner by which cystic fibrosis

From the Division of Reproductive Genetics, Department of Obstetrics and Gynecology, The University of Tennessee, Memphis,[a] and the Law, Medicine, and Ethics Program, Boston University Schools of Medicine and Public Health.[b]
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Reprint requests: Sherman Elias, MD, Department of Obstetrics and Gynecology, Room E-102, University of Tennessee, Memphis, 853 Jefferson Ave., Memphis, TN 38163.
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transmembrane conductance regulator is involved in regulating ion conductance across the apical membrane of epithelial cells is unclear.[7] Nonetheless, identification and characterization of the cystic fibrosis gene offer strategies for increasing the accuracy of cystic fibrosis prenatal diagnosis beyond that possible by linkage analysis alone.

Genetics

In the United States white population of European ancestry (excluding Ashkenazic Jews and Hispanics), about 75% of the mutations in cystic fibrosis patients correspond to a three base pair deletion. This deletion results in the loss of a phenylalanine residue at amino acid position 508 (designated ΔF_{508}) from amino acid coding region 1480 of the cystic fibrosis gene.* From a study of 200 non-Ashkenazic white families in which a family member had cystic fibrosis, Lemna et al.[9] determined that 58.2% of individuals affected with cystic fibrosis were homozygous for the ΔF_{508} mutation, 33.7% were heterozygous for this mutation, and 8.0% were homozygous for the absence of this mutation. The frequency of the ΔF_{508} mutation varies among different ethnic groups. For example, in Spanish and Italian populations the mutation accounts for only 49% and 43% of cystic fibrosis mutations, respectively.[10] The 3 base pair deletion appears to be much less common in the U.S. Ashkenazic Jewish population, in whom approximately 30% of cystic fibrosis mutations occur.[9] The frequency of cystic fibrosis in black Americans is about 1 in 17,000 births[11]; however, the proportion of cystic fibrosis cases that result from the ΔF_{508} mutation in this population has not yet been established.

If a 1 in 2500 frequency of cystic fibrosis in the U.S. non-Ashkenazic white population is assumed, the likelihood of detecting an individual as a cystic fibrosis carrier can be determined from the various mutation detection rates (Table I). If, for example, 75% of cystic fibrosis mutations are detectable by DNA analysis, the likelihood that an individual with a negative test is a carrier is reduced from an a priori risk of 1 in 25 to a new risk of 1 in 99. If the DNA of one parent is positive for a mutation but the DNA of the other parent is negative, the risk that the couple will have a child with cystic fibrosis is (1, or known heterozygote)(1/99)(1/4), or 1/396. If DNA of neither parent tests positive for a mutation, the risk of having a child with cystic fibrosis is (1/99)(1/99)(1/4), or about 1/39,200. Contemplating population-based screening necessitates deriving the likelihood of detecting couples who are actually cystic fibrosis carriers; this can be calculated as a function of the mutation detection rate (Table II). At a 75% mutation detection rate, for example, both partners will be identified as carriers in 56.3% of the cases; in 37.5% of couples only one of the partners will be identified as a cystic fibrosis carrier; in 6.2% neither partner will be identified as a cystic fibrosis carrier.[9]

A number of clinical scenarios can be envisioned for the United States non-Ashkenazic population, if a cystic fibrosis mutation detection rate of 75% is assumed.

Previous child affected with cystic fibrosis. *Both parents are heterozygous for ΔF_{508}, with or without the availability of the affected child.* Couples who have a child with cystic fibrosis have a 1 in 4 recurrence risk in each subsequent pregnancy, unless there is the rare occurrence of a new mutation. If a child with cystic fibrosis is homozygous for the ΔF_{508} mutation and the parents are heterozygous for the same mutation, prenatal diagnosis by direct mutation analysis with the use of either chorionic villi or amniotic fluid cells is the method of choice in terms of speed and reliability. Identification of such a child can be expected in 0.75×0.75, or 56.3% of cases. The ΔF_{508} mutation analysis provides almost 100% accuracy in identifying an affected fetus and does not require DNA from a previously affected child.

Parents have discrepant mutations in the cystic fibrosis gene; DNA from the affected child is available. The affected child could be a compound heterozygote for the ΔF_{508} mutation; i.e., one parent could contribute a chromosome 7 with the ΔF_{508} mutation and the other parent could contribute a chromosome 7 with a different mutation in the cystic fibrosis gene. A child also could be affected with cystic fibrosis as a result of each parent's contributing a chromosome 7 with a mutation in the cystic fibrosis gene other than the ΔF_{508} mutation. In both situations prenatal diagnosis with a high degree of accuracy can still be offered if DNA from the affected child is available. A combination of ΔF_{508} mutation analysis and restriction fragment length polymorphism linkage analysis is used in the former circumstance, whereas linkage analysis alone is used in the latter.

Parents have discrepant mutations in the cystic fibrosis gene; DNA from the affected child is not available. If one parent carries the ΔF_{508} mutation and the other parent does not, fetal DNA can be analyzed to determine if the fetus did or did not inherit the mutation from the ΔF_{508} carrier parent. If not, the fetus is unaffected. If the ΔF_{508} carrier parent *did* transmit the cystic fibrosis gene to the fetus, there is a 1 in 2 risk that the fetus is affected. Under these circumstances amniotic fluid microvillar-intestinal-enzyme analysis would be particularly useful in predicting fetal cystic fibrosis status. Enzyme analysis

*In the context of this article, the term *mutation analysis* refers to abnormalities of the cystic fibrosis gene itself, which is demonstrable by DNA analysis (e.g., the ΔF_{508} mutation). By contrast, the term *linkage analysis* refers to restriction fragment length polymorphism DNA markers that are linked to the cystic fibrosis gene but usually are not within the actual gene. Thus restriction fragment length polymorphism analysis only indirectly provides information concerning the presence or absence of the cystic fibrosis mutation.

carries an estimated false-positive rate of 2% and a false-negative rate of 8%.[9]

Close relative with cystic fibrosis. An unaffected sib of an individual with cystic fibrosis has a 2 in 3 likelihood of being a cystic fibrosis heterozygote and a 1 in 3 likelihood of being homozygous normal. An uncle or aunt of an individual with cystic fibrosis has a 1 in 2 likelihood of being a cystic fibrosis heterozygote and a 1 in 2 likelihood of being homozygous normal. A first cousin of an individual with cystic fibrosis has a 1 in 4 likelihood of being a cystic fibrosis heterozygote and a 3 in 4 likelihood of being homozygous normal. Thus, if an individual has a close relative with cystic fibrosis (sib, niece, uncle, or first cousin), that individual should be offered carrier testing with mutation analysis or linkage analysis. If the parent who has a close relative with cystic fibrosis is indeed found to be a carrier, mutation analysis of the spouse is indicated. If both parents are determined to be carriers, their risk for having offspring with cystic fibrosis is 1 in 4; prenatal diagnosis would be appropriate. If one parent carries the ΔF_{508} (presumably the high-risk parent) and the other parent does not carry the ΔF_{508} mutation, the couple's risk for having a child with cystic fibrosis is 1 in 396. If the ΔF_{508} carrier parent *did* transmit the cystic fibrosis gene to the fetus, there is still only a 1 in 198 risk that the fetus is affected. Under these circumstances prenatal diagnosis could not provide an unequivocal determination of fetal status. Even if microvillus intestinal enzyme analysis were used, on the basis of known false-positive (2%) and false-negative (8%) rates for this test,[9] Bayesian calculations show that an abnormal result would yield a 19% probability that the fetus is affected. Thus there is an 81% probability that microvillus intestinal enzyme analysis would represent a false-positive result.

Population-based cystic fibrosis carrier screening. In the United States non-Ashkenazic white population about 75% of heterozygotes have the ΔF_{508} mutation. Therefore, as shown in Table II, only about 50% of those couples in which both partners are cystic fibrosis carriers can now be identified by screening for the ΔF_{508} mutation. If additional cystic fibrosis mutations were discovered and if, for example, 95% of carriers could be detected, then 90% of couples at risk would be identified (Table II). It was initially hoped that the remaining 25% of cystic fibrosis mutations would be relatively few in number so that most, if not all, cystic fibrosis carriers could be identified efficiently.[1] Unfortunately, this has not proved to be the case; more than 20 additional mutations have already been identified, each of which is individually rare. Thus, if population-based cystic fibrosis screening is undertaken, one partner in about 1 in 15 couples would be identified as a carrier of the ΔF_{508} mutation and the other partner would not carry this mutation. However, failure of the other partner to have the ΔF_{508} mutation does not exclude that partner's being a cystic fibrosis carrier for a different cystic fibrosis mutation but rather merely reduces the likelihood from 1 in 25 to 1 in 99 (Table I). In these couples the risk of having a child with cystic fibrosis is about 1 in 400, but prenatal diagnosis cannot presently provide an unequivocal determination of fetal cystic fibrosis status even with the combination of amniotic fluid microvillus intestinal enzyme testing and restriction fragment length polymorphism linkage analysis.

Table I. Cystic fibrosis–related risks after mutation analysis for carrier status

Cystic fibrosis mutations detectable (%)	*Carrier risk for person with negative test*	*Risk of cystic fibrosis in offspring of couples tested*	
		One parent positive	*Neither parent positive*
0	1/25.5	NA	1/2,500
70	1/82.7	1/331	1/27,400
75	1/99	1/396	1/39,200
80	1/124	1/494	1/61,000
85	1/165	1/661	1/109,200
90	1/246	1/984	1/242,100
95	1/491	1/1964	1/964,400

NA, Not applicable. (Reproduced with permission from Lemna et al. N Engl J Med 1990;322:291-6.)

Table II. Potential for detection of couples at risk with use of population-based screening for carriers

Cystic fibrosis mutations detectable (%)	*Detection of carrier status (%)*		
	Both parents	*One parent*	*Neither parent*
70	49.0	42.0	9.0
75	56.3	37.5	6.2
80	64.0	32.0	4.0
85	72.3	25.5	2.2
90	81.0	18.0	1.0
95	90.3	9.5	0.2

(Reproduced with permission from Lemna et al. N Engl J Med 1990;322:291-6.)

Public policy for carrier screening

In March 1990 the National Institutes of Health Workshop on Population Screening for the Cystic Fibrosis Gene[12] was convened to make suggestions about the introduction of cystic fibrosis screening into medical practice. The group concluded that pilot programs to investigate research questions in the delivery of population screening for cystic fibrosis are "urgently needed." The National Institutes of Health panel merely listed the following five guidelines that are already widely accepted as applicable to any widespread

population screening. We believe that each merits elaboration in the context of cystic fibrosis screening.

Screening should be voluntary and confidentiality must be assured. The National Academy of Sciences recommended in 1975 that "participation in a genetic screening program should not be mandatory by law, but should be left to the discretion of the person to be tested or, if a minor, of the parents or legal guardian."[13] The President's Commission on Bioethics strongly endorsed this recommendation and noted: "Some individuals are likely to choose not be screened for cystic fibrosis, and their ability to make that choice must be safeguarded."[14]

Confidentiality enhances the trust implicit in the doctor-patient relationship, and medical information should not be disclosed to third parties without informed consent. The President's Commission stated: "Because of the potential for misuse as well as unintended social or economic injury, information from genetic testing should be given to people such as insurers or employers only with the explicit consent of the person screened."[14] We can foresee no circumstances under which confidentiality should be breached in cystic fibrosis carrier screening programs. Even if an individual who is identified as a carrier refuses to allow this information to be conveyed to a sibling, the a priori risk of that sibling's having a child with cystic fibrosis is $<1\%$.

Screening requires informed consent. Providers of screening services have an obligation to assure that adequate education and counseling are included in the program. Autonomy is an illusion if the individual does not understand to what he or she is being asked to consent. Accordingly, there is a need to develop prescreening educational material that adequately explains, in lay terms, the benefits of screening, as well as the limitations and risks of screening, such as loss of insurability and adverse psychosocial effects. This information must, of course, be made available to the individual *before* consent for screening is sought. In addition, professional counseling about the significance of positive screening results and reproductive options must be available when results are made known to the individual.

The goal of genetic screening and counseling is "to make people into informed decision makers about their genetic constitution, to the extent it is relevant to choices about their own well-being or that of their family."[14] The success of population-based cystic fibrosis carrier screening will depend on the practicing medical community's becoming knowledgeable and supportive of the entire process, as well as on educating and involving the public. Before population carrier screening begins, informational campaigns at national and local levels must be in place to educate the public about the objectives and availability of screening. It has been estimated that if 3 million couples per year who contemplate having children were screened and if those couples were counseled for 10 minutes before the test and at-risk couples were counseled for an hour after the test, 651,000 hours of counseling would be required to meet the screening-generated demand.[15] Cystic fibrosis screening of this limited population would require more than 17 weeks per year from each of the approximately 1000 certified genetic counselors and clinical geneticists.[15] The alternative is that primary providers such as obstetrician-gynecologists have the requisite knowledge and counseling skills.

As the number of possible genetic screening tests proliferates, it will be necessary to devise new strategies to educate and help individuals to decide to use specific screening tests. These tests may ultimately involve such interventions as interactive videotapes that can be played at home and in physician's waiting rooms, as well as the use of specially trained nonphysician counselors in clinics and schools.

The National Institutes of Health panel concluded that the current difficulties with cystic fibrosis screening (i.e., only 75% of carriers are detectable) "would be substantially reduced if testing could detect at least 90 to 95% of carriers." Although there is certainly a degree of arbitrariness about any cutoff point for inclusive detection, this degree of inclusiveness (90% to 95%) seems reasonable if one of the major benefits of screening is excluding individuals from the "at-risk" category. First, without a screening process that meets this degree of inclusiveness, the amount of reassurance one can provide to a couple is substantially reduced. Second, the result may be to foster both fear and stigmatization. Third, at a detection rate of 75%, the costs of population screening are very high as compared with the benefits. Wilfond and Fost,[15] for example, estimate that at that rate 11,100 couples would have to be screened to detect 10 couples at risk. If 80% of those families chose prenatal diagnosis, two (25%) would be expected to have an affected fetus. If half (one) of those families with an affected fetus terminate the pregnancy, one of two cystic fibrosis births would be avoided. At $200 for screening and counseling per couple, the cost of avoiding one cystic fibrosis birth in this scenario is $2.2 million. Even if the detection rate were increased to 95% and the cost were decreased to $70 per couple, the cost would still be $1 million per cystic fibrosis birth avoided, and this does not take into account costs incurred by effects that result from error or stigmatization.[15]

On the other hand, individual members of the public may feel that a lower cutoff (namely, 57%) is acceptable for individual testing (versus population screening) and may reject the National Institutes of Health panel's perhaps somewhat paternalistic conclusion. A flexible ap-

proach thus seems prudent and reasonable at this point. If a highly motivated individual or couple without a family history requests cystic fibrosis carrier testing, the current state-of-the-art (as set forth above) should be carefully explained by the physician, either directly or by a genetic counseling center. After this explanation, if the individual still wants to be tested, it is not unreasonable for the physician to comply with this request.

Quality control of all aspects of the laboratory testing, including systematic proficiency testing, is required and should be implemented as soon as possible. Accurate screening and counseling will require reliable test results. It is thus important that national standards for laboratory testing be established, that the laboratories involved in performing cystic fibrosis testing be monitored in some reasonable manner, and that the results of this monitoring be made available to the medical profession and the public.[16]

There should be equal access to testing. We agree with the National Institutes of Health panel and others that routine screening of the general population is premature at this point. However, if successful pilot programs demonstrate that the benefits outweigh the costs and that counseling can be done effectively, the question will be who should be screened? The National Institutes of Health panel stated: "the most appropriate group for population screening is reproductive aged individuals," and primary health providers were defined as "the optimal setting." Society is currently wrestling with the issues of whether cystic fibrosis screening should be covered by public insurance and of what the minimum benefit package should consist. Unless the cost of the test can be reduced from its present $150 to $200, it may be difficult to justify population screening covered by private insurance, even more difficult to justify population screening covered by public insurance, and even more difficult yet to justify screening black Americans. Additional cost and benefit data will be needed to make this assessment.

Ideally cystic fibrosis screening would be performed before pregnancy, at a time when couples still have such options as artificial insemination, adoption, or not having children at all. Some couples might choose to be screened but even when identified as cystic fibrosis carriers elect to take their chances with the knowledge that there is "only" a 25% risk of having an affected child. However, screening adults for heterozygosity is generally useful only if prenatal diagnosis is available. At present, it can be assumed that couples in which both partners are identified to be heterozygotes for cystic fibrosis would usually proceed to prenatal diagnosis with the intent of pregnancy termination if the fetus is found to be affected.

Inevitably, this will become an increasingly difficult choice for some couples. This is not only because many affected children cope with their disease admirably and even live for four decades, but also because with the cloning of the cystic fibrosis gene and the determination of the gene product cystic fibrosis transmembrane conductance regulator, there is new optimism that either somatic gene therapy or medical therapy targeted toward the underlying abnormalities will provide a "cure" or significant amelioration for patients with cystic fibrosis in the foreseeable future.

Before they undergo amniocentesis or chorionic villus sampling, couples who are cystic fibrosis carriers will need knowledgeable counseling by experts in cystic fibrosis who can accurately detail, in unbiased fashion, the probable clinical course of the disease, as well as the medical progress being made in its treatment. At some point it is to be hoped that cystic fibrosis will no longer be a reason to consider pregnancy termination. At present, however, the final decision regarding pregnancy termination must rest with the accurately counseled couple.

The obligations of obstetrician-gynecologists

Screening that is offered as part of more generalized health care services (e.g., through high school, college, or family planning clinics) would encourage preconceptual cystic fibrosis carrier screening. For most couples, however, the first opportunity for carrier screening takes place after a pregnancy has occurred. The primary responsibility for providing cystic fibrosis carrier screening will rest with obstetrician-gynecologists, as does the provision of other types of genetic screening, such as tests recommended because of advanced maternal age or screenings for maternal serum α-fetoprotein, hemoglobinopathies, or Tay-Sachs disease.

One important recommendation of the National Institutes of Health panel was that cystic fibrosis screening "should be offered to all individuals and/or couples with a family history of cystic fibrosis." On the other hand, for many of the reasons already detailed, the group recommended against screening those individuals with a negative family history "under the current circumstances." This recommendation is consistent with recent professional organization statements. The Committee on Obstetrics: Maternal and Fetal Medicine of the American College of Obstetricians and Gynecologists has reviewed and agreed with a policy statement written by the American Society of Human Genetics, which states the following:

First, carrier testing should be offered to couples in which either partner has a close relative affected with [cystic fibrosis]. Second, one or a few federal, foundation, or privately supported pilot programs should be conducted as soon as possible in order to gather more data regarding laboratory, educational and coun-

seling aspects of screening. Third, there is an immediate need for centralized quality control of laboratories conducting these tests. Fourth, it will be appropriate to begin large-scale population screening in the foreseeable future, once the test detects a larger proportion of [cystic fibrosis] carriers and more information is available regarding the issues surrounding the screening process. Until that time it is considered premature to undertake population screening.

Finally . . . it is the position of the American Society of Human Genetics that routine [cystic fibrosis] carrier testing of pregnant women and other individuals is NOT yet the standard of care in medical practice.[17]

Current "standard of care" by obstetrician-gynecologists should include a genetic history and should offer (not require) prenatal diagnostic testing to couples who have had a previous child with cystic fibrosis.[18] The real question is how we decide whether cystic fibrosis screening should be routinely recommended for individuals of reproductive age who have a negative family history. The President's Commission on Bioethics,[14] for example, stated: "Public screening programs should not be implemented until they have first demonstrated their value in well-conducted pilot studies." The National Institutes of Health panel concurred with this recommendation in the following statement:

Pilot programs investigating research questions in the delivery of population screening for CF carriers are urgently needed. These programs should address clearly defined questions including effectiveness of educational materials, level of utilization, laboratory aspects, counseling issues, costs, and beneficial and deleterious effects of screening.

We agree. Good science and solid educational and counseling strategies must be in place before cystic fibrosis screening is routinely offered to those with a negative family history. Unlike maternal serum α-fetoprotein screening, in which a primary impetus for introduction as a routine test was to avoid malpractice suits,[19] cystic fibrosis carrier screening should be deemed "standard of care" only after the appropriate scientific committees (e.g., the Committee on Obstetrics: Maternal and Fetal Medicine of the American College of Obstetricians and Gynecologists) have reviewed all relevant data, including those on educational strategies, and have concluded that offering such screening will probably provide more benefit than harm to individuals and couples. This will help ensure that cystic fibrosis carrier screening will be introduced in a coordinated and effective manner.

Under the influence of aggressive private companies that are marketing cystic fibrosis tests, individual practitioners may "jump the gun" and offer routine cystic fibrosis screening. The medical profession can still be effective in managing the introduction of cystic fibrosis testing and screening into clinical practice. This is important, because ad hoc approaches to genetic screening, such as those used to identify sickle cell anemia, Tay-Sachs disease, maternal serum α-fetoprotein, and now cystic fibrosis screening, are ultimately unsatisfactory.[15] As the human genome initiative continues, tens, if not hundreds of new genetic screening tests may compete for introduction into routine clinical practice. It is past time to begin to develop guidelines for determining how we should judge each such new test, which tests should be routinely offered, and who should pay for each of them. Current procedures in which professional associations such as the American College of Obstetricians and Gynecologists and expert panels at the National Institutes of Health make recommendations do not contemplate or include effective participation by the public in such decisions. Because the public will ultimately be asked to accept or refuse such screening and because the ultimate objective of all such screening is to benefit the public, we recommend that all such groups take steps to involve the public in their deliberations about which screening tests physicians should routinely offer to their patients.

Addendum

Since submission of this manuscript, the cystic fibrosis mutation detection rate has increased to about 85%. As shown in Table II, 72.3% of cystic fibrosis carrier couples could now be identified.

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Determinants of perinatal outcome in pregnancy-induced hypertension with absence of umbilical artery end-diastolic frequencies

Fiona M. Fairlie, MD,[a] Michael Moretti, MD,[b] James J. Walker, MD,[a] and Baha M. Sibai, MD[b]

Glasgow, Scotland, and Memphis, Tennessee

Forty-three pregnancies that were complicated by pregnancy-induced hypertension and either absence of umbilical artery end-diastolic frequencies ($n = 32$) or reversal of umbilical artery end-diastolic frequencies ($n = 11$) were reviewed. The incidence of perinatal mortality and morbidity was similar for the two Doppler patterns. Perinatal survival was highly dependent on the gestational age when hypertension first appeared. Presentation at ≥30 weeks' gestation was associated with a perinatal survival rate of 86%. Presentation at <30 weeks' gestation was associated with a perinatal survival rate of 38% ($p < 0.005$). Pregnancy-induced hypertension that presented before 30 weeks was more often associated with a 5-minute Apgar score <7 ($p < 0.005$) and a nonreactive nonstress test ($p < 0.05$) compared with pregnancy-induced hypertension that presented at or beyond 30 weeks. For pregnancies that presented before 30 weeks, the only difference between perinatal survivors ($n = 11$) and perinatal deaths ($n = 18$) was a higher incidence of birth weight at or below the 10th percentile among deaths ($p = 0.02$). (Am J Obstet Gynecol 1991;164:1084-9.)

Key words: Absence of end-diastolic frequencies, Doppler ultrasonography

The absence of end-diastolic frequencies in the umbilical artery Doppler waveform is a normal finding before 18 weeks' gestation.[1] After 20 weeks' gestation, absence of end-diastolic frequencies is not normal and has been shown to be associated with high perinatal mortality and morbidity rates.[2,3] The reported perinatal mortality rate that is associated with this Doppler finding ranges from 17% to 89%.[3-5] Reversal of end-diastolic frequencies is the most severe umbilical artery waveform abnormality.[6] It has been suggested that almost all fetuses with reversal of end-diastolic frequencies are asphyxiated.[7]

The optimum management of pregnancies with absence of end-diastolic frequencies or reversal of end-diastolic frequencies is controversial. Some have recommended immediate delivery in all cases,[4] and others suggest delivery in selected cases according to gestational age and the presence or absence of other risk factors.[7] The small size of studies to date, the inclusion of a variety pregnancy complications, and fetuses with lethal abnormalities have made it difficult to determine the most appropriate method of management of this Doppler pattern.

In this article, we report on our experience of absence of end-diastolic frequencies and reversal of end-diastolic frequencies in pregnancy-induced hypertension. The purpose of our study was to identify factors that correlate with perinatal outcome and may prove useful in determination of the appropriate management of pregnancy-induced hypertension that is complicated by absence of end-diastolic frequencies or reversal of end-diastolic frequencies.

Patients and methods

Two centers participated in this study, the E.H. Crump Hospital and Perinatal Center in Memphis, Tennessee and the Glasgow Royal Maternity Hospital in Scotland. Between January 1, 1986, and July 1, 1989, 43 pregnancies that were complicated by hypertension were found to have absence or reversal of umbilical artery end-diastolic frequencies. Thirty-two pregnancies were complicated by preeclampsia[8] (American Col-

From the Perinatal Research Center, Glasgow Royal Maternity Hospital,[a] and the E.H. Crump Women's Hospital and Perinatal Center, Memphis.[b]
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Reprint requests: Dr. F. M. Fairlie, Perinatal Research Center, Glasgow Royal Maternity Hospital, Rottenrow, Glasgow G4 ONA, Scotland.
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lege of Obstetricians and Gynecologists definition).[8] Eleven pregnancies were complicated by pregnancy-induced hypertension without proteinuria.

In Glasgow, Doppler studies were performed with a Doptek continuous wave system (Doptek, Chichester, England). A 150 Hz high-pass filter was used to eliminate low-frequency signals that are caused by vessel wall movement. In Memphis, Doppler recordings were obtained with an Ultramark 9 (Advanced Technology Laboratories, Inc., Bothell, Wash.) with continuous-wave and pulsed-wave Doppler ultrasonography and a 50 Hz high-pass filter.

Umbilical artery waveforms were recorded from free-floating loops in the midportion of the umbilical cord. A diagnosis of absence of end-diastolic frequencies or reversal of end-diastolic frequencies was accepted when this Doppler pattern was demonstrated at three separate sampling sites. Doppler signals were recorded in the absence of fetal breathing and trunk movements. Uteroplacental flow-velocity waveforms were recorded on the right and left sides with continuous-wave Doppler ultrasonography with the technique described by Schulman et al.[9] A normal uteroplacental pattern was defined by a mean systolic/diastolic ratio of less than 2.7.[9] Since the patients in this study were recruited from two distinct population groups it was considered inappropriate to use normal Doppler data from either Glasgow or Memphis. For this reason the normal range that was published by Schulman et al.[9] was used. In both participating centers, Doppler measurements were concealed from the obstetricians who were responsible for patient management.

All pregnancies that were studied had ultrasonographic studies dated from before 24 weeks' gestation. Amniotic fluid volume was classified as normal, reduced, or oligohydramnios by subjective assessment and measurement of the largest cord-free pocket. Oligohydramnios was defined as the absence of cord-free pocket with a vertical diameter of ≥2 cm. The nonstress test was classified as reactive if there were at least two accelerations of the fetal heart rate of 15 beats/min amplitude and 15 seconds' duration within a 20-minute recording period.[10] If two accelerations were not evident after a 20-minute recording period, the fetus was stimulated by either vibratory or acoustic stimulation.[11] A fetal heart rate pattern that did not become reactive after fetal stimulation was classified as nonreactive. If a nonreactive pattern was associated with spontaneous fetal heart rate decelerations, it was classified as decelerative. When the result of a nonstress test was nonreactive, a biophysical profile was performed with the technique described by Manning et al.[12]

Both centers that participated in this study attempted to prolong pregnancy unless there was evidence of fetal and/or maternal distress.[13-16] Antihypertensive therapy was administered to 38 patients (88% of study group). Maternal indications for delivery included uncontrollable hypertension, eclampsia, thrombocytopenia (platelet count ≤100,000 per cubic millimeter) and the HELLP syndrome (*h*emolysis, *e*levated *l*iver enzymes, and *l*ow *p*latelets) as defined according to Sibai et al.[17] Elevated liver enzymes were not an indication for delivery unless the elevation was accompanied by another complication. The principal fetal indications for delivery were either a nonreactive nonstress test with spontaneous decelerations or ultrasonic evidence of fetal growth retardation with reduced amniotic fluid volume or oligohydramnios. In four cases, the indication for delivery was a biophysical profile score ≤4 of 10 on two occasions that were separated by a period of 6 hours. Since a biophysical profile was only performed on patients with a nonreactive nonstress test (i.e., 20 patients), it was not considered in the analysis of results.

The following perinatal outcome data were collected; birth weight, birth weight percentile for gestation, mode of delivery, 5-minute Apgar score, umbilical venous blood pH, partial pressure of oxygen, and partial pressure of carbon dioxide, and the incidence of intraventricular hemorrhage, necrotizing entercolitis, and seizures. Birth-weight percentile was defined according to the growth charts of Brenner et al.[18] for the Memphis population and according to those of Forbes and Smalls[19] for the Glasgow population. Venous blood gases in the cord were classified as normal or abnormal according to the normal reference ranges of Nicolaides et al.[20] All neonates were followed up until they left the hospital. In this study, perinatal deaths included stillbirths, deaths within the first month of life, and deaths of infants who died before the age of 12 months without leaving the neonatal unit.

Statistical analysis was performed with either the Student *t* test or Fisher's exact test.

Results

There were 23 survivors and 20 perinatal deaths. The perinatal deaths included 8 intrauterine fetal deaths, 11 neonatal deaths, and 1 infant death. The infant death occurred at 149 days of age. Six survivors are currently younger than 12 months old. All six showed normal physical and neurologic development at 9-month follow-up. Four survivors have bronchopulmonary dysplasia.

The incidence of cesarean section for the total study group was 79%. Of the 35 live-born infants, 34 were delivered by cesarean section, and in 32 cases, delivery was before the onset of labor. One patient who presented at or beyond 30 weeks' gestation delivered vaginally.

There were 32 pregnancies with absence of umbilical

Table I. Pregnancies with absence of umbilical artery end-diastolic frequencies compared with pregnancies with reversal of end-diastolic frequencies

Pregnancies	*ABEDF*	*REDF*
No.	32	11
Survivors	18	5
Neonatal deaths	9	3
Intrauterine deaths	5	3
Mean gestation at delivery (wk, mean ± SD)	30 ± 3.5	29 ± 2.5
Delivery because of fetal indications	19	8
Birth weight ≤10th percentile	24	11
5 min Apgar score <7	9	2
Necrotizing entercolitis	3	0
Seizures	1	0
Intraventricular hemorrhage	5	3

ABDEF, Absence of end-diastolic frequencies; *REDF,* reversal of end-diastolic frequencies.

artery end-diastolic frequencies and 11 pregnancies with reversal of end-diastolic frequencies. Table I compares perinatal mortality and morbidity for these two Doppler groups. There was no difference in any of the outcome parameters that were studied.

Preliminary data analysis indicated that there was a dramatic improvement in perinatal survival if pregnancy gestation was at or beyond 30 weeks' gestation when hypertension first presented. Since the main purpose of the study was to identify determinants of perinatal outcome in the presence of absence of end-diastolic frequencies or reversal of end-diastolic frequencies, pregnancies in which hypertension presented before 30 weeks' gestation were compared with pregnancies in which hypertension presented at or beyond 30 weeks' gestation. This comparison is shown in Table II. The initial impression of a significantly higher perinatal survival if hypertension presented at or beyond 30 weeks' gestation was confirmed ($p < 0.005$). All intrauterine fetal deaths occurred in pregnancies in which hypertension presented before 30 weeks' gestation. Seven patients were delivered of infants because of maternal indications, and all presented before 30 weeks' gestation ($p \leq 0.05$). These pregnancies resulted in four survivors and three neonatal deaths. Pregnancy losses at or beyond 30 weeks' gestation were confined to two neonatal deaths after deliveries at 30 weeks' gestation. The birth weights of these neonates were 870 gm and 614 gm, and death occurred at 8 days and 3 days after delivery, respectively. Both infants had severe respiratory distress and intraventricular hemorrhage.

In Table II, fetal monitoring test results were the ultimate test results before delivery. In all cases, the interval between the last monitoring test and delivery did not exceed 4 days. In a comparison of pregnancies that presented before 30 weeks' gestation with those that presented at or beyond 30 weeks' gestation, there was no difference in the incidence of reversal of end-diastolic frequencies. A reactive nonstress test was more common at or beyond 30 weeks ($p < 0.05$). There was no difference in the incidence of decelerative nonstress test pattern. Both abnormal amniotic fluid volume (i.e., reduced amniotic fluid volume or oligohydramnios) and birth weight at or below the 10th percentile were significantly more common at or beyond 30 weeks' gestation ($p < 0.05$). There was a higher incidence of 5-minute Apgar scores <7 in pregnancies that presented before 30 weeks' gestation ($p < 0.005$), but no difference in the incidence of neonatal morbidity. Uteroplacental data were obtained for 27 patients (63% of the study group). There was no difference in a comparison of the incidence of abnormal uteroplacental Doppler studies in pregnancy-induced hypertension that presented before 30 weeks with pregnancy-induced hypertension that presented at or beyond 30 weeks.

Only two pregnancies that presented before 30 weeks' gestation delivered at or beyond 30 weeks' gestation. Pregnancies that presented before 30 weeks' gestation and resulted in perinatal survival ($n = 11$) were compared with pregnancies that presented before 30 weeks' gestation and resulted in perinatal death (either neonatal death $n = 10$ or intrauterine fetal death $n = 8$). The same characteristics as shown in Table II were compared. The incidence of birth weight at or below the 10th percentile was significantly less among survivors (5 of 11) as compared with perinatal deaths (16 of 18, $p < 0.05$; Fisher's exact test). No other differences were detected.

Thirty-two infants were delivered by elective cesarean section. Umbilical venous blood for gas analysis was obtained at delivery in 17 cases. The results are shown in Table III. In a comparison of survivors with neonatal deaths, there was no difference in the incidence of acidosis, hypoxemia, or hypercapnia at delivery. Eight survivors and five neonatal deaths showed absence of end-diastolic frequencies, and three survivors and one neonatal death showed reversal of end-diastolic frequencies. Two of the three survivors with reversal of end-diastolic frequencies were acidotic, hypoxemic, and hypercapnic, and one was acidotic and hypercapnic. The neonatal death with reversal of end-diastolic frequencies was acidotic, hypoxemic, and hypercapnic.

Serial Doppler data were obtained for 16 survivors and 12 perinatal deaths. The recorded duration of absence of end-diastolic frequencies ranged from 1 to 10 days for survivors and 1 to 18 days for perinatal deaths. The recorded duration of reversal of end-diastolic fre-

Table II. Pregnancies that presented before 30 weeks' gestation compared with pregnancies that presented at or beyond 30 weeks' gestation

Pregnancies	*Before 30 weeks*	*At or beyond 30 weeks*
No.	29	14
Survivors	11	12*
Neonatal deaths	10	2
Intrauterine fetal deaths	8	0†
REDF	8	3
Birth weight ≤10th percentile	21	14†
Delivery because of fetal indications	14	13*
Delivery because of maternal indications	7	0†
Reactive NST	1	4*
Decelerative NST	10	6
Normal AFV	11	1†
Normal uteroplacental doppler study‡	3	1
5 min Apgar score <7§	11	0*
Necrotizing enterocolitis§	3	0
Intraventricular hemorrhage§	5	3
Seizures§	1	0

REDF, Reversal of end-diastolic frequencies; *NST,* nonstress test; *AFV;* amniotic fluid volume.

*$p < 0.005$ (Fishers' exact test).

†$p < 0.05$ (Fishers' exact test).

‡Uteroplacental Doppler studies were obtained in 15 pregnancies before 30 weeks and in 12 pregnancies at or beyond 30 weeks.

§Only refers to survivors and neonatal deaths.

quencies ranged from 1 to 4 days for survivors and 1 to 2 days for deaths. Two pregnancies were followed up for more than 14 days. One resulted in an intrauterine fetal death at 29 weeks. The other was delivered at 29 weeks and neonatal death occurred at 24 days. In five pregnancies, absence of end-diastolic frequencies preceded a change in the nonstress test. The interval between the first recording of absence of end-diastolic frequencies and the change in the nonstress test ranged from 1 to 14 days. In three cases, the nonstress test changed from reactive to decelerative, and in two cases, the change was from nonreactive to decelerative. Four of these infants were delivered by elective cesarean section because of fetal distress and all survived. The fifth member of this group died in utero.

Table III. Umbilical vein blood gas analysis: Survivors compared with neonatal deaths

Results of blood gas analysis	*Survivors**	*Neonatal deaths**
No.	11	6
Acidosis	10	5
Hypoxemia	6	5
Hypercapnia	10	6

*Only infants that were delivered by elective cesarean section are included in this analysis.

Comment

Most previous reports of absence of end-diastolic frequencies or reversal of end-diastolic frequencies have been limited to fewer than 30 cases and have included a variety of pregnancy complications and fetal abnormalities. Our study is unique by virtue of its size and by its restriction to one specific pregnancy complication with the exclusion of fetal abnormalities.

It has been suggested that reversal of end-diastolic frequencies is a more ominous sign, in terms of perinatal outcome, than absence of end-diastolic frequencies.[6] This was not evident in our study group. The perinatal mortality that was associated with absence of end-diastolic frequencies was 44% as compared with 54% for mortality that was associated with reversal of end-diastolic frequencies. The incidence of perinatal morbidity and gestational age at delivery was also similar for these two groups. In our experience of pregnancy-induced hypertension that was associated with absence of end-diastolic frequencies or reversal of end-diastolic frequencies, gestational age at presentation was a more important determinant of perinatal outcome than was the severity of the umbilical artery Doppler abnormality.

The purpose of our study was to identify characteristics of pregnancies with pregnancy-induced hypertension and absence of end-diastolic frequencies or reversal of end-diastolic frequencies, which correlated with perinatal outcome and may prove helpful in antepartum management. Gestation at presentation of hypertension was a major determinant of perinatal mortality and morbidity (Table II). Presentation before 30 weeks was associated with a significantly lower incidence of survivors and with significantly more intra-

uterine fetal deaths as compared with pregnancy-induced hypertension that occurred at or beyond 30 weeks. Nonstress test reactivity and Apgar scoring are partly dependent on gestational age. However, it is difficult to explain the significantly lower incidences of reactive nonstress tests and Apgar scores ≥7 in pregnancies that presented before 30 weeks on the basis prematurity alone. In addition, maternal complications were more common among pregnancies that presented before 30 weeks' gestation. These differences are in keeping with a more severe disease process if pregnancy-induced hypertension presented before 30 weeks.

Table II shows the incidence of delivery because of fetal indications to be higher after 30 weeks. However, if intrauterine fetal deaths are taken into account, then the incidence of delivery because of fetal indications was the same before 30 weeks as at or beyond 30 weeks. It may be postulated that at gestations before 30 weeks, obstetricians decided that the risk of neonatal morbidity and mortality was too high to justify delivery. Consequently, the asphyxiated fetus died in utero. However, it is not possible to conclude from this study that immediate delivery for pregnancy-induced hypertension that presents before 30 weeks will improve perinatal outcome.

It is unlikely that the method of delivery had a major impact on perinatal outcome since 97% of live-born infants were delivered by cesarean section. For pregnancies that presented before 30 weeks' gestation, infants that died during the perinatal period were more likely to be small for gestational age (below the 10th percentile of birth weight for gestation) compared with survivors. Although placental function is not the only determinant of perinatal survival and birth-weight percentile, it is an important factor. In our study group, the incidence of abnormal uteroplacental Doppler results was high—85% of all pregnancies studied. We found no correlation between the uteroplacental Doppler waveform and perinatal outcome. This finding agrees with the conclusions of Trudinger and Cook[21] who studied uteroplacental Doppler waveforms in severe pregnancy-induced hypertension.

Gas analysis of umbilical cord blood that was obtained at cordocentesis correlates well with samples that were obtained at elective cesarean section.[22] We compared cord venous blood gas analysis after elective cesarean section for survivors and neonatal deaths and found no difference in the incidence of acidosis, hypoxemia, or hypercapnia. It has been suggested that virtually all fetuses with umbilical artery reversal of end-diastolic frequencies are asphyxiated and that cordocentesis may be of value in assessment of the well-being of the previable fetus with absence of end-diastolic frequencies.[7] We found a high incidence of abnormal gases in association with both absence of end-diastolic frequencies and reversal of end-diastolic frequencies, regardless of perinatal outcome. This would suggest that blood gas analysis by cordocentesis may benefit only a very small proportion of fetuses with absence of end-diastolic frequencies or reversal of end-diastolic frequencies, that is, those with normal blood gases. In a study of umbilical blood samples that were obtained at cordocentesis from fetuses that were small for gestational age, Nicolaides et al.[20] concluded that umbilical venous hypoxemia or hyperlactacidemia were good determinants of uteroplacental insufficiency. It is interesting that Table III shows a trend (nonsignificant) toward less hypoxemia among survivors as compared with neonatal deaths.

Johnstone et al.[3] described the outcome of 24 pregnancies with absence of umbilical artery end-diastolic frequencies. Twenty were delivered by cesarean section, and 22 were below the 5th percentile. There were four deaths—a much lower perinatal mortality rate than that reported in other studies of absence of end-diastolic frequencies. Four pregnancies continued for 3 to 6 weeks after the finding of absence of end-diastolic frequencies. Only one of these four had signs of fetal distress, and only two were delivered vaginally. The duration of absence of end-diastolic frequencies in these four pregnancies is interesting. In our study, the duration of absence of end-diastolic frequencies ranged from 1 to 18 days in the two pregnancies that were followed up for more than 2 weeks, and both resulted in perinatal death. The exact duration of absence of end-diastolic frequencies or reversal of end-diastolic frequencies is unknown unless daily Doppler examinations are performed. However, the impression from our study and previous studies is that fetuses vary in their ability to tolerate the vascular pathologic conditions that are responsible for absence of end-diastolic frequencies.[3, 23] Our experience confirms the observation, which was reported by others,[3, 23] that absence of end-diastolic frequencies may precede a change in the nonstress test by a variable time interval.

We would agree with other authors that absence or reversal of umbilical artery end-diastolic frequencies is associated with a high risk of adverse perinatal outcome but is not an indication for immediate delivery. In our specific study group of pregnancies that were complicated by pregnancy-induced hypertension, perinatal mortality and morbidity were almost entirely confined to hypertension that presented before 30 weeks' gestation. For pregnancies that presented beyond 30 weeks' gestation, delivery that was based on traditional tests of fetal monitoring (i.e., nonstress tests and ultrasonic assessment of fetal growth and amniotic fluid volume) was associated with 86% perinatal survival. It may be postulated that for pregnancies that present before 30 weeks' gestation, perinatal outcome could be im-

proved by prolonging gestation. However, this is only possible if the fetus is healthy and if maternal condition is stable.

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Intrauterine transfusion treatment of nonimmune hydrops fetalis secondary to human parvovirus B19 infection

Vicken Sahakian, MD,[a] Carl P. Weiner, MD,[a] Stanley J. Naides, MD,[b] Roger A. Williamson, MD,[a] and Laura L. Scharosch, AB[b]

Iowa City, Iowa

Congenital human parvovirus B19 infection presented in this case as nonimmune hydrops fetalis that resulted from aplastic anemia at 20 weeks' gestation. Intravascular transfusion therapy led to resolution of the hydrops and a term delivery of an appropriately grown neonate. (AM J OBSTET GYNECOL 1991;164:1090-1.)

Key words: Nonimmune hydrops fetalis, parvovirus, cordocentesis

Human parvovirus B19 was first identified in 1975 and has subsequently been shown to be the cause of several diseases, including aplastic crisis in patients with chronic hemolytic anemia, erythema infectiosum, a chronic rheumatoid-like arthritis, and chronic bone marrow suppression.[1] The most common clinical presentation of parvovirus in an infected fetus with symptoms is nonimmune hydrops. The hydrops can result either from aplastic anemia with high-output cardiac failure or from myocarditis. Intrauterine fetal therapy with digoxin for the latter condition has been attempted but has been only partially successful.[2] Theoretically, treatment of an aplastic crisis should be straightforward. Recently, Peters and Nicolaides[3] reported the reversal of B19 hydrops in two fetuses after antenatal transfusion. We wish to report a third case, which supplies additional biochemical and biophysical parameters that shed further light on the involved pathophysiology.

Case report

A 23-year-old primigravid woman was referred to the University of Iowa Fetal Diagnosis and Treatment Unit in September 1989 at 21 weeks' gestation after fetal hydrops was found during a routine ultrasonographic examination approximately 1 week earlier. The patient had recently begun work at a children's day-care center. She denied signs or symptoms that would be compatible with a viral illness during the pregnancy.

From the Fetal Diagnosis and Treatment Unit, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology,[a] and the Division of Rheumatology, Department of Internal Medicine,[b] University of Iowa College of Medicine.

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Reprint requests: Carl P. Weiner, MD, Department of Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, IA 52242.

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A level II ultrasonographic examination revealed a fetus with biometric parameters that were consistent with 21 weeks' gestation. Anhydramnios and frank anasarca that were characterized by subcutaneous edema, fetal ascites, and cardiomegaly with a pericardial effusion were detected. In addition, the lateral cerebral ventricles were modestly dilated.

A cordocentesis was performed (Table I). The pancytopenia was consistent with a viral infection as was the total level of immunoglobulin M (IgM) of 46 mg/dl (upper limits of normal, 10 mg/dl). The umbilical venous pressure was elevated and consistent with congestive heart failure. An intravascular transfusion was performed through the umbilical vein on an emergent basis and the hematocrit partially corrected to 22%. The transfusion was repeated 48 hours later, at which time the final hematocrit was 39%. The umbilical venous pressure had normalized by the time of the second transfusion.

A maternal B19 serologic test was performed with an antibody-capture enzyme-linked immunosorbent assay as previously described.[1] Both IgM and immunoglobulin G (IgG) anti-B19 antibodies were positive in maternal serum on Sept. 6, 1989. Electron microscopic examination of fetal blood and nonheparinized fetal ascites by negative staining revealed viral particles that were morphologically consistent with B19 as previously described.[2] Polymerase chain reaction amplification of B19 deoxyribonucleic acid was performed with B19-specific primers, and Southern analysis was performed with a B19-specific probe as previously described.[1] B19 deoxyribonucleic acid was detected in fetal serum and ascites on Sept. 6, 1989, and in fetal serum 2 weeks later, on Sept. 19, 1989. There was no evidence of viremia in maternal serum when it was tested on Sept. 6, 1989, and on Sept. 20, 1989.

Two weeks after the transfusion therapy, the fetal hematocrit was stable and the reticulocyte index had risen to 4.9% (Table I). There was clear improvement in the liver function studies. Significantly, the total level of IgM had already declined to 15 mg/dl.

The fetus was closely monitored. All ultrasono-

graphic abnormalities resolved over the next 4 weeks. Delivery occurred at term in the referring hospital. Cord serum that was obtained at delivery was negative for anti-B19 IgM but positive for IgG anti-B19 antibodies. At the time of delivery, fetal viremia was no longer detected. The 7-pound 8-ounce male infant was in good condition and was discharged with his mother. At 1 year of age his development remains normal.

Comment

A causal relationship between B19 infection and hydrops fetalis with subsequent fetal death has been documented.[2] The antenatal diagnosis and palliative treatment of parvovirus B19 nonimmune hydrops fetalis in association with myocarditis was first reported in 1989.[2] Although the hydrops resolved with direct fetal digitalization, that fetus died of overwhelming myocarditis. This case, in which the hydrops was associated with severe anemia, appears to be the third fully documented case of antenatal diagnosis with treatment by intrauterine transfusion, complete resolution of hydrops, and a viable outcome. A fourth case has been reported in preliminary form, but documentation of fetal infection was limited to the demonstration of maternal anti-B19 IgM antibodies and B19-like particles in fetal serum.[4]

Early in gestation, the fetus is particularly vulnerable because of a shortened erythrocyte life span and hyperplastic erythropoiesis. The pathogenesis of the hydrops frequently appears to be secondary to the cytopathic infection of red blood cell precursors with arrest of red blood cell production, resultant severe anemia, and subsequent congestive heart failure. The severe anemia that was present in our case and associated with a low reticulocytic index and a normal bilirubin level suggests a similar pathogenesis as opposed to hemolysis. The very low white blood cell and platelet counts probably reflect suppression of the myeloid and thrombopoietic cell lines in addition to the erythropoietic ones. The elevated liver function tests are consistent with hepatic dysfunction, including hepatitis (unpublished norms from the Iowa Fetal Diagnosis and Treatment Unit).

Two weeks after transfusion, the hematocrit was stable and the reticulocyte count doubled. This remarkable recovery offers hope that if the fetus survives the acute infectious episode, survival is possible. In one reported case the hydropic fetus had begun to mount a reticulocytic response when treated and may have recovered without therapy.[3]

In the case presented here, the elevated umbilical venous pressure is consistent with high-output cardiac failure. Its correction with partial transfusion supports hypoxic myocardial dysfunction as at least part of the mechanism. The marked decline of the total IgM antibodies within 2 weeks suggests that the fetal immune system may be too immature to sustain anti-B19 IgM antibody production when the antigenic stimulation occurs before 20 weeks' gestation. This decline further demonstrates that a search for congenital infection should not rely on total IgM measurements.

Table I. Intrauterine transfusion therapy

Laboratory test	*Before transfusion*	*2 wk after transfusion*	*Normal**
Hematocrit (%)	5.8	39.8	30.8-38.4†
White blood cell count (No./mm³)	0.4	2.7	3.11 ± 0.7†
Platelets (No./mm³)	31,000	113,000	255,000 ± 51,000
Reticulocyte count (%)	2.3	4.7	12.2 ± 3.6†
Lactate dehydrogenase (IU/L)	925	375	202 ± 30†
Aspartate aminotransferase (IU/L)	52	12	13 ± 3†
Total bilirubin	1.1	2.2	1.4 ± 0.2†
IgM (mg/dl)	46	15	<10
Umbilical venous pressure (mm Hg)	14	2	<10
Umbilical venous Po_2 (mm Hg)	38	40	>38†

*University of Iowa Fetal Diagnosis and Treatment Unit, 95% or mean ± SD.

†For gestational age.

The complete resolution of hydrops after fetal transfusion also suggests that fetal B19 infection is a self-limited infection that will eventually be followed by recovery if the fetus survives the acute initial insult. This case report of a B19-infected fetus suggests that intrauterine transfusion may be used as a last resort for the treatment of such severely affected hydropic fetuses.

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Attitudes on the ethics of abortion, sex selection, and selective pregnancy termination among health care professionals, ethicists, and clergy likely to encounter such situations

Mark I. Evans, MD,[a] Arie Drugan, MD,[b] Sidney F. Bottoms, MD,[a] Lawrence D. Platt, MD[c] Charles A. Rodeck, MD,[d] Manfred Hansmann, MD,[e] and John C. Fletcher, PhD[f]

Detroit, Michigan, Haifa, Israel, Los Angeles, California, London, England, Bonn, Federal Republic of Germany, and Charlottesville, Virginia

The ethical attitudes of health care providers toward abortion, sex selection, and selective termination of normal and anomalous fetuses in singleton or multiple pregnancies were evaluated by questionnaires distributed to members of the American Society of Human Genetics, the International Fetal Medicine and Surgery Society, the Society of Perinatal Obstetricians, ethicists, and clergy. Demographic characteristics of respondents exhibited a preponderance of men (76%), age >40 (68%), and of United States residents (82%). Seventy-nine percent of respondents were in the medical profession. Approximately half of the respondents were Protestant, the rest being evenly distributed among Catholic, Jewish, and other religions. Acceptance of abortion for social indication varied by religion and gestational age but not by religious conviction, age, country, or gender of respondent. First-trimester abortion of a normal singleton pregnancy was considered more acceptable than selective termination of normal fetuses in multifetal gestations. Sex selection was considered unethical by most respondents. Selective termination was deemed ethically appropriate in quadruplets or multifetal gestations of more than five fetuses and in multiple pregnancies bearing one anomalous fetus. In the latter situation, acceptance increased with the severity of fetal anomalies and decreased from the first to the third trimester. The medical specialty of respondents was the only independent factor strongly associated with acceptance of selective termination by trimester, indication, and number of fetuses. Acceptance of selective termination among health care professionals appears to reflect not only perceptions of procedure-related risks and benefits in the index pregnancy but also individual training and religious beliefs. (AM J OBSTET GYNECOL 1991;164:1092-9.)

Key words: Abortion, sex selection, selective termination, ethics

The recent debate after the United States Supreme Court decision on *Webster vs Reproductive Health Services* has reopened legal questions concerning a woman's ability to obtain an abortion. In the next several years, state laws may diverge dramatically from the protection that *Roe vs Wade* gave from interference by states with a woman's choice.

The ethics of controversial topics such as abortion per se, sex selection, and selective termination must be studied and shaped independently from the legal and political processes. We undertook to survey those professionals likely to encounter pregnant patients who might be candidates for difficult choices such as whether to have an abortion for fetal abnormalities or a selective termination for either an abnormality or a multifetal pregnancy. We then compared their views on the ethical appropriateness of such procedures against their baseline view of abortion for nonobstetric-nonmedical indications ("social abortion") and abortion for sex selection. The study questions were: Do these professionals approve of abortion or termination of one or more fetuses under any circumstances? What do their views suggest about the actual shape of moral reasoning in practice?

These data were all obtained before *Webster* and therefore may be used in the coming years as a benchmark for change in ethical views as changes in the law may force changes in medical practice.

Material and methods

Questionnaires testing attitudes about the acceptability of social abortion, abortion for lethal fetal anomalies, abortion for significant but not lethal fetal anom-

From the Center for Fetal Diagnosis and Therapy, Departments of Obstetrics and Gynecology, Molecular Biology, and Genetics, and Center for Molecular Biology, Hutzel Hospital/Wayne State University, Detroit,[a] the Department of Obstetrics and Gynecology "A," Rambam Medical Center, Haifa,[b] the Department of Obstetrics and Gynecology, University of California Los Angeles School of Medicine (Cedars Sinai),[c] the Department of Obstetrics and Gynecology, University College Hospital, London,[d] the Department of Obstetrics and Gynecology, Universitats-Frauenklinik Bonn, Bonn,[e] and the Biomedical Ethics Program, Department of Medicine, University of Virginia School of Medicine, Charlottesville.[f]

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Reprint requests: Mark I. Evans, MD, Director, Division of Reproductive Genetics, Hutzel Hospital/Wayne State University, 4707 St. Antoine Boulevard, Detroit, MI 48201.

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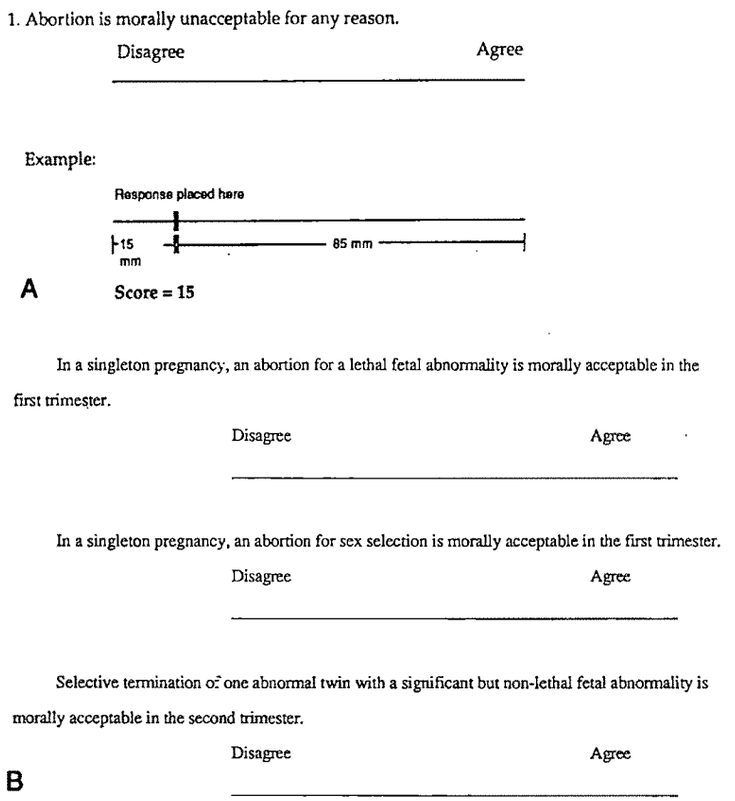


Fig. 1. Example of statements **(A)** and method of response **(B).**

alies, sex selection, and selective termination in twins and higher-order multifetal pregnancies were circulated to members of the American Society of Human Genetics (ASHG), the International Fetal Medicine and Surgery Society (IFMSS), the Society of Perinatal Obstetricians (SPO), and ethicists and clergy with known involvement in biomedical issues. Questions were then stratified by the trimester of pregnancy.

We assumed that demographics may influence the response to specific statements. Independent variables used therefore were age (<40, 40 to 49, or ≥50 years), specialty (maternal-fetal medicine, medical [not maternal-fetal medicine], genetics, and nonmedical professionals), country (United States vs others), gender, religion (Protestant, Jewish, Catholic, and others), and religious conviction (the degree that the responder considered himself or herself religious).

Respondents were asked to note degrees of agreement with statements by marks on a 100 mm line, with complete disagreement scored as 0 and complete agreement scored as 100. Scores were determined with a millimeter rule as the distance of the mark from the zero score (Fig. 1, *A*). A few example questions are also shown (Fig. 1, *B*).

To minimize the effects of extreme values ("outliers"), scores were transformed to a nominal scale of 0 to 3; 0 is complete disagreement and 3 is complete agreement with the statements. Multivariate analyses of variance for repeated measures (software, BMDP statistical package, Santa Monica, Calif.) were used to analyze scores. Trimester and number of fetuses were repeated-measures adjusted as within-subject factors. Characteristics of the respondents were between-subject factors and selection on the basis of a priori interest and data reduction with the use of stepwise log-linear analysis. To minimize the effect of multiple comparisons, $p < 0.001$ was considered significant.

Results

The demographic characteristics of the 737 questionnaire respondents' analyzed are shown in Table I. Respondents were predominantly men, over age 40, and therefore would not personally face the dilemma of carrying a multiple pregnancy.

The first statement posed the moral acceptability of social abortion. Most of the respondents (74.1%) strongly disagreed with a statement that abortion in the first trimester is morally unacceptable. The only variable related to this response was religion ($p < 0.001$); first-trimester abortion was more accepted among Jewish respondents than among Moslems or Protestants (in this order) and least accepted among Catholics (Fig.

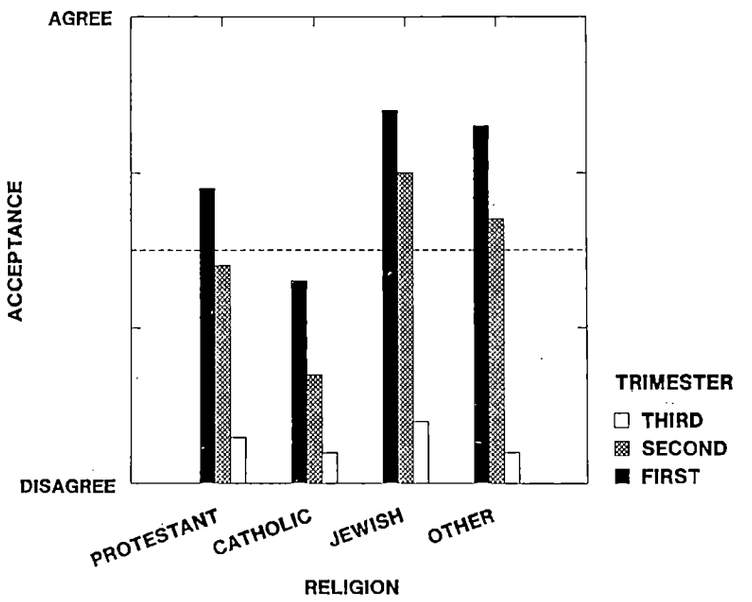


Fig. 2. Acceptance of social abortion, by religion, in first, second, and third trimesters.

Table I. Demographic characteristics of respondents

Characteristic	%
Age (N = 723)	
<40 yr	39
40-49 yr	33
>50 yr	27
Specialty (N = 734)	
Maternal-fetal medicine	43
Genetics	22
Medical	14
Nonmedical	22
Religion (N = 720)	
Protestant	46
Catholic	22
Jewish	18
Other	15
Religious conviction (N = 734)	
Not religious	13
Moderately religious	59
Very religious	27
Country (N = 732)	
United States	82
Other	18
Gender (N = 731)	
Male	76
Female	24

2). In contrast, sex selection of singleton pregnancies in the first, second, or third trimester was found unacceptable by most of the respondents; (67.2%, 74.6%, and 92.5%, respectively). Differences could not be documented by group, age, religion, sex (Fig. 3), specialty, or country of origin. The association with religious conviction was, however, marginally significant (p = 0.0012).

We then questioned the acceptability of selective termination of one or more presumably "normal" fetuses in multifetal gestation. Acceptance of selective termination in this situation increased with the number of fetuses (from two to four) and decreased with advancing gestational age (Fig. 4). Social terminations of singleton pregnancies were perceived as being more acceptable than selective termination for social reasons in twins, triplets, or quadruplets. Agreement with a woman's right to interrupt a normal pregnancy for social reasons and the relative risks of selective termination to remaining fetuses (as compared to risk of multiple gestation) may explain these findings.

Analyses of the factors associated with these results show that the group and specialty of respondents strongly influenced acceptance of selective termination in association with the number of fetuses and trimester ($p < 0.0001$). Acceptance was highest among geneticists and lowest among non-medical ethicists and clergy (Fig. 5). The effects of religion and religious conviction were documented only in relation to trimester, not to number of fetuses. Selective termination for quadruplets was more acceptable among professionals in maternal-fetal medicine than among other groups. The highest acceptance of selective termination was among Jewish respondents; the lowest was among Catholics. A weak association with gestational age was also documented for country and age, whereas the association between these factors and number of fetuses was not significant. The gender of respondent did not influence acceptability of selective termination as evaluated by trimester or by number of fetuses (Table II).

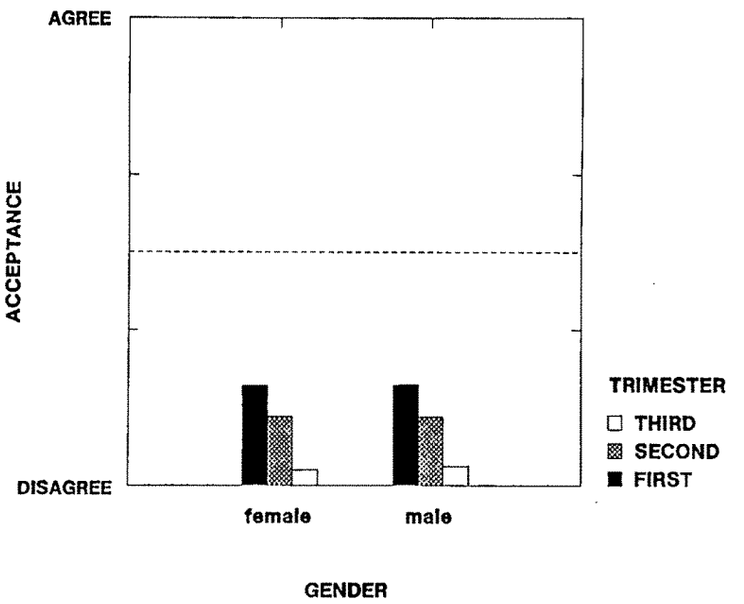


Fig. 3. Acceptance of sex selection, by gender of respondent, in first, second, and third trimesters.

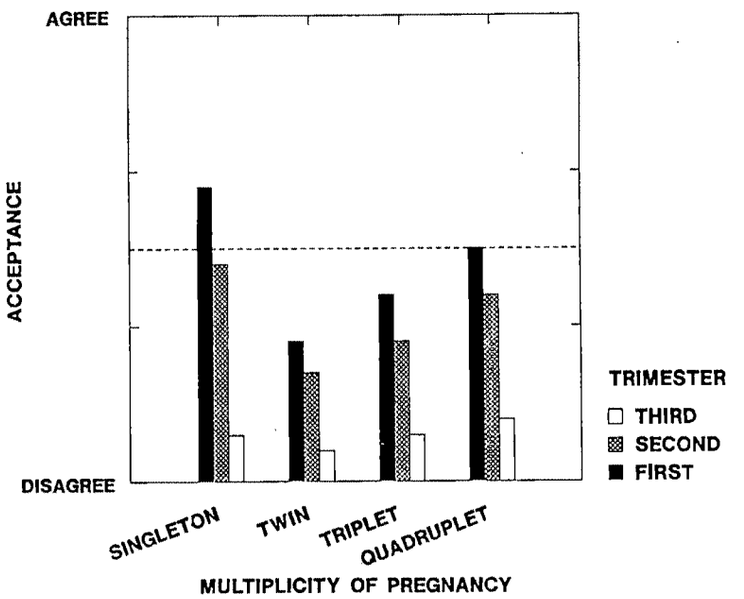


Fig. 4. Acceptance of singleton abortion and selective termination, according to fetal number, in first, second, and third trimesters.

The effects of the indication for termination (social, significant nonlethal anomaly, and lethal anomaly) in twins and singletons also were evaluated. The acceptability of abortion in singletons increased with the severity of anomalies involved and decreased with advancing gestation. The same trend was observed in twins, although the acceptability of selective termination of the affected twin in the second and third trimesters was lower than for singletons. Overall, acceptance of selective termination was strongly associated with the trimester of pregnancy, indication for selective termination, and fetal number (whether it be a singleton or twin gestation).

Specialty was the only independent variable affecting acceptance of selective termination in relation to indication, trimester, and number of fetuses (Table III).

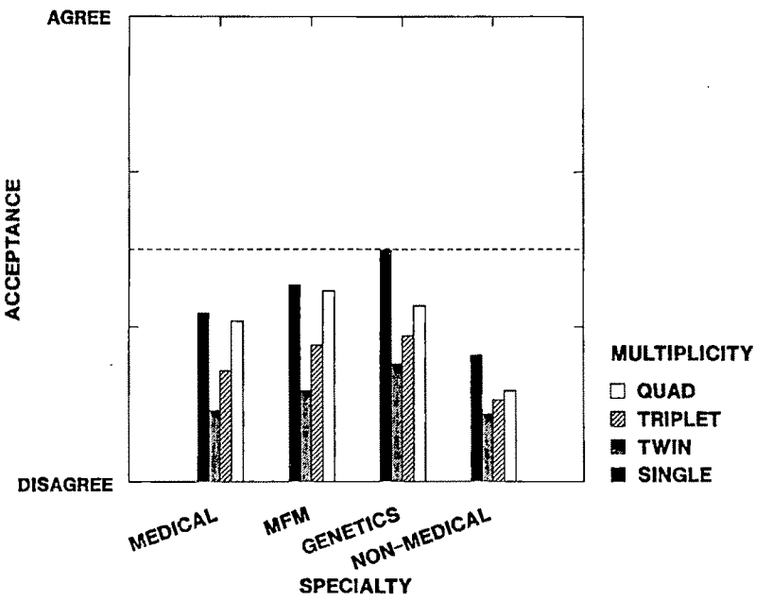


Fig. 5. Acceptance of selective termination according to fetal number and specialty of respondent.

The highest acceptance was among geneticists; the lowest was among nonmedical professionals. A significant difference, according to indication, was also found between United States and non–United States respondents. Acceptability of social terminations was higher in United States respondents whereas acceptability of termination for anomalies was higher for non–United States respondents. The trimester or number of fetuses did not affect this response. Likewise, gender did not influence acceptability of selective termination by indication, trimester, number of fetuses, or any of the interactions within.

Most respondents considered that in multiple pregnancy with two fetuses there is a 95% chance of survival without significant morbidity, and in triplets there is a 90% chance. A 50% chance of intact survival was considered by most as the chance in quadruplets. Most respondents considered that quintuplets have less than a 5% chance of survival with acceptable morbidity for all fetuses. No relationship could be documented between these responses and age, gender, group, specialty, religion, religious conviction, or country.

Quadruplets were elected as the optimal number of fetuses to offer selective termination in the first or second trimester (67.4% and 62.6% of respondents, respectively). The optimal number of remaining fetuses after the procedure was two (67.8%). No interactions were evident between these choices and the independent variables. The minimal number and maximal number of fetuses to be left in utero after selective termination were 2 and 3, respectively. These choices were identical whether the selective termination was performed in the first or second trimester of pregnancy.

The question of safety and the need for centralization of services for selective termination was also addressed by respondents. Only 6.3% of respondents strongly disagreed with the statement that selective termination is a safe procedure in the first trimester, whereas 36.5% of respondents already strongly believed in the safety of this procedure. However, second-trimester procedures were considered less safe. Respondents who completely disagreed and completely agreed with the safety of second-trimester selective termination were 12.1%, and 17% respectively. No relationship could be documented between these responses and age, sex, country, specialty, religion, or religious conviction. More than half of the respondents (52.2%) strongly agreed that selective termination should be performed in a small number of centers with considerable experience in this technique. Again, none of the independent variables was significantly related to this response.

Comment

Our data show the following:

1. Acceptability of abortion in singleton pregnancies increases with the severity of anomalies and decreases with advancing gestation. Acceptability of selective termination of one abnormal twin follows the same general rules but is lower than for singletons in all categories. This may be explained by considerations of risk conferred by the procedure to the normal remaining twin.

2. Acceptability of abortion according to trimester

(but not to number of fetuses and indication) was also determined by religion. A weaker association with the degree of religious conviction was also found. This may indicate that the point in pregnancy when fetal rights become compelling may differ by religion—from conception through the point that a premature fetus is believed to have a reasonable chance of viability, about 24 gestational weeks at present. Acceptance of selective termination in the third trimester, when viability is established, was uniformly low.

3. Specialty was the only independent variable strongly associated with acceptability of termination according to trimester, indication, and number of fetuses. The variation may indicate that acceptance of selective termination may be based on more day-to-day exposure to neonatal problems seen by physicians who specialize in birth defects. However, this conclusion has to be taken with extreme caution. There are many potential confounders to this point, including the underlying assumptions and biases of physicians as to why they went into their respective specialties.

4. Sex selection is not widely accepted among health professionals, ethicists, and clergy. A majority considered that sex selection was not an ethically acceptable reason for offering prenatal diagnosis in otherwise uncomplicated situations. There was relative agreement about the unacceptability of sex selection among all specialties, religions, sex, age, or country of the respondents.

5. Respondents accept selective termination as an ethical procedure in cases of multiple gestation (four or more fetuses) or in cases of fetuses affected by serious anomalies. The acceptance of a woman's prerogative in reproductive decisions and the possible risk to remaining fetuses from the selective termination procedure cause higher acceptance of abortion in singleton pregnancies than of reduction of fetal number. High-risk pregnancy specialists were more concerned about the risks of a multifetal pregnancy having poor outcome than were nonmedical personnel.

6. Age, gender, and country or origin did not significantly influence acceptability of selective termination. However, the higher acceptance of social abortion in the United States as opposed to higher acceptance for severe or lethal fetal anomalies by non–United States respondents may indicate a different set of values in these subgroups.

Thus it seems that, although decisions may be influenced by the training or religious beliefs of some, most health care professionals in this study view selective termination as an ethically acceptable procedure, provided that the benefits that can be expected to outweigh the risks. Both the benefits and risks associated with selective termination are, however, still poorly defined at present. The former can be learned by comparing

Table II. Acceptability of selective termination for social indications

	Trimester	*No. of fetuses*
Age	$p < 0.001$	NS
Specialty	$p < 0.0001$	$p < 0.0001$
Religion	$p < 0.0001$	NS
Religious conviction	$p < 0.0001$	$p < 0.001$
Country	$p = 0.0001$	NS
Gender	NS	NS

Table III. Acceptability of selective termination according to indication (social, nonlethal anomaly, or lethal fetal anueploidy)

	Indication	*Trimester*	*No. of Fetuses*
Age	NS	NS	NS
Specialty	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Religion	NS	$p < 0.0001$	NS
Religious conviction	NS	$p < 0.0001$	$p < 0.001$
Country	$p < 0.0001$	NS	NS
Gender	NS	NS	NS

the number of infants born without the risk of prematurity after selective termination with the outcome in several published series of multifetal gestations.[1-6] Increased risks for preeclampsia, postpartum hemorrhage, thromboembolic phenomena, and premature delivery are also reported for mothers with multifetal conceptions.[7] Prolonged bed rest and hospitalization have significant psychological, social, and economic implications. Despite treatment, 15% to 28.6% combined perinatal and infant mortality has been reported with triplets, 38.4% with quadruplets, and 70% with sextuplets.[5, 6] With birth weights <1000 gm the perinatal mortality is 50%, and more than 20% of survivors will suffer moderate to severe handicaps.[8] Thus only about 30% of infants from multifetal pregnancies weighing <1000 gm born in level III hospitals will be considered intact survivors.

The clinical experience and ethical considerations in selective termination procedures have been the subject of several recent reports.[9-12] The presumed benefit of this procedure is to enhance the chances of survival and decrease morbidity among the remaining fetuses. Whether this goal is achieved by selective termination is not clear at present as the world's experience is still only a few hundred cases. The risks could be minimized by limiting the procedure to be performed in only a few centers by individuals with extensive experience in ultrasonographically guided techniques. However, even in experienced hands, the pregnancy loss rate after the procedure is reported to be in the range of

20% to 30%.[12, 13] It is imperative that centers that do perform selective termination share their experiences openly.

The desperate situation imposed by the diagnosis of multifetal pregnancy in patients treated for infertility or severe anomalies incompatible with meaningful life in one twin may cause patients to volunteer for experimental, innovative therapy and sign the consent form without experiencing the true meaning of "informed" consent. It is important to counsel patients on the yet unknown risks and uncertain outcomes of selective termination. It is most importantly that efforts should be extended to minimize the need for this procedure by careful monitoring of ovulation induction and by limiting the number of embryos or gametes transferred in in vitro fertilization or in gamete intrafallopian transfer. However, even with these precautions, some multifetal pregnancies will still occur. Moreover, severe anomalies affecting only one fetus in multifetal gestations will continue to happen occasionally. The choice in these cases seems to be between interruption of the entire pregnancy or the abortion of some fetuses so that others may survive with minimal morbidity. We believe that choosing selective termination is a morally acceptable, albeit experimental procedure. According to our data, this belief is shared by many other health care professionals and by some religious leaders, especially if selective termination is offered in the first trimester.

The data about sex selection, as compared with other indications for abortion, reveal much about the moral views of the respondents. Also, if the views of the respondents are similar to the moral views in society as a whole, these data prompt points of reference in the discussion of public policy on abortion in the post-*Webster* debates. After *Webster*, new debates will occur in each state about the best public policy on abortion and whether it should be restricted or unrestricted.

Our views

Abortions done to protect the woman's life are morally acceptable in virtually every ethical tradition. Abortions done to protect a woman's health or respect her consent to pregnancy are the next consideration. Furthermore, many ethical traditions, and the moral views of the respondents to this study, support the moral justification for abortion of a fetus diagnosed with a severe genetic disorder.

Even in the third trimester, in addition to the indication protection of the life and health of the mother, some have argued that pregnancies can be ethically (and usually legally) ended when there is diagnostic evidence of the following: (1) a condition that is incompatible with life after delivery, (2) the death of the infant is imminent, (3) attempts at treatment would be inhumane, and (4) the only option open would be "comfort care."[14, 15]

A counter argument, however, is that not all reasons to become pregnant, to have an abortion, or to alter the number of fetuses in a pregnancy are morally acceptable. To the extent possible, public policy ought to consider both sides of the situation in abortion or selective termination choices.

The views of our respondents are a good index of the ethical "limits" that should be placed on practitioners in assisting with abortions or selective terminations. Our respondents agreed strongly that abortion for sex selection was morally objectionable, although not all respondents made this judgment about the procedure in the first trimester. We believe but cannot extrapolate from the data that there is a distinction between what is unethical and what should be illegal. Abortion for gender selection violates equality in a radical way. Also, gender is not a disease, and to abort for gender is a precedent for eugenics. *Eugenics* means a deliberate attempt to alter characteristics (or avoid them) that have little or nothing to do with disease. If the eugenics of genetic manipulation is to be avoided in the future, when the human genome is mapped, every precedent for eugenics in this generation should be prevented. Ideally, when there is no problem or a small problem, there is not a need for a law. In fact there have been a few preliminary attempts at the state level (e.g., Pennsylvania, California) to outlaw sex selection, for abortion in toto (Louisiana), but we believe such attempts are hopelessly intertwined with politics and do not encourage good medical care, let alone have the capability to be enforced. No one knows the actual incidence of abortion for sex selection in the United States. Some studies show some openness to this idea, as does this study. Openness does not mean that it actually occurs in practice, although a few cases certainly do in fact occur each year.

Another unethical possibility, but a remove one, is to begin a pregnancy with the prior intent of: (1) abortion to donate fetal tissue for therapy to another, (2) selling fetal tissue after abortion to another, or (3) abortion of the fetus if it is not a compatible bone marrow or organ donor for another. Since it is already against Federal law to *sell* fetal tissue and a National Institutes of Health commission has recommended that decisions to direct donations of fetal tissue before abortion be prohibited,[16] it is likely that state laws will incorporate provisions 1 and 2. Provision 3 is likely to be so rare as not to present a public policy problem. However, we recommend that practitioners not cooperate with requests for prenatal diagnosis and/or abortion related to intentions to define the fetus in advance as a source of organ, marrow, or tissue donation for others. This intent violates the personhood of the future individual, in the sense that he or she would be viewed primarily as a means to an end for another person.

Selective termination in pregnancy (of an affected twin or in cases of multifetal pregnancies) is not "abor-

tion." Abortion literally means the emptying of the human uterus. However, if new abortion laws are to be passed, it is likely that these interventions in pregnancy will become subjects of debate. Participants in the debate from the scientific community are urged to familiarize themselves with the facts of these procedures. The views of our respondents reflect that they believe it to be moral to attempt selective termination of a genetically affected twin and in a multifetal pregnancy that follows infertility treatments. However, they object on moral grounds to selective termination of a normal twin for social or economic reasons. The risks of delivery of normal twins do not, in their view, include dangers or complications sufficient to justify the risks of intervention to eliminate one twin. The entire pregnancy could be lost. Also, there are considerations of the loss of general respect for human life that surround unrestricted selective termination of one twin.

If a public policy on abortion and procedures for selective termination in pregnancies is to be debated, the views of the respondents to this study probably have wide support in the society for a moral position that approves some abortions and procedures and rejects others. A sound abortion law must protect the choices of women to terminate pregnancies to which they did not consent or plan (rape, incest, unplanned pregnancies), those that threaten their lives or health, and those that they desire to terminate after diagnosis of a genetic or chromosomal disorder. The legal "cutoff" periods for these choices must be carefully thought through, in terms of what is known about fetal neural development and the timing of midtrimester procedures for genetic diagnosis.

A public policy could include prohibitions against abortions that are sought only for sex choice and abortions to benefit others, either by sale of tissue for use or by donation. However, public policy should protect the rights of parents (with the expert help of physicians) to make difficult choices in difficult situations.

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The significance of amniotic fluid volume during intrapartum fetal acoustic stimulation

Albert P. Sarno, Jr., MD, Major, MC, USA
Los Angeles, California, and Honolulu, Hawaii

The relationship of intrapartum fluid volume to the fetal response to acoustic stimulation was investigated in 112 patients who were in the latent phase of labor. Amniotic fluid volume appears to play an insignificant role in the genesis of fetal heart rate decelerations that occur after fetal acoustic stimulation, regardless of the amount of amniotic fluid present. (Am J Obstet Gynecol 1991;164:1100-1.)

Key words: Amniotic fluid volume, fetal heart rate, fetal acoustic stimulation

The fetal response to externally applied sound stimuli has generated a great deal of clinical research interest recently, although many questions remain to be answered.

Fetal heart rate (FHR) decelerations have been noted to follow a reactive fetal response in 20% to 25% of patients.[1] The cause of this pattern is uncertain but is thought to be due to transient cord compression as a result of the fetal startle response. Recently, Menashe and Sherer[2] have suggested that fetal acoustic stimulation may be contraindicated in cases of oligohydramnios because of an increased risk for cord compression that would lead to prolonged bradycardia. However, data that describe the significance of amniotic fluid volume during fetal acoustic stimulation have not yet been reported.

This study examines the effect of amniotic fluid volume on the fetal response to acoustic stimulation in patients who are in the latent phase of labor.

Patients and methods

The study population consisted of 112 patients who presented in the latent phase of labor to the labor and delivery floor of Women's Hospital at the Los Angeles County/University of Southern California Medical Center or Tripler Army Medical Center in Honolulu, Hawaii. Inclusion criteria were: (1) term gestation ≥36 completed weeks, (2) singleton pregnancy, (3) vertex presentation, and (4) latent phase of labor (≤4 cm dilatation).

Amniotic fluid volume was assessed with a semiquantiative technique, the amniotic fluid index.[3]

Electronic fetal monitoring was instituted and was followed by performance of fetal acoustic stimulation with a 3-second sound pulse with a fetal acoustic stimulator (model 146, Corometrics Medical Systems, Inc., Wallingford, Conn.), sound level 82 dB at 1 m in air. If no acceleration of the FHR was noted within 1 minute, an additional pulse was administered to a maximum of three pulses, each 1 minute apart. A reactive response was defined as one or more accelerations of the FHR that were 15 beats/min from baseline and persisted for 15 seconds. An FHR deceleration was defined as a drop in FHR of at least 15 beats/min that persisted for 15 seconds or more within 1 minute of sound stimulation. A nonreactive response was defined as failure to elicit a qualifying acceleration after any of three separate stimuli and for 10 minutes after the last stimulus. Care was taken to avoid performance of acoustic stimulation during or immediately after a uterine contraction to avoid periods of transient fetal hypoxia and to standardize the technique.

Data were collected and analyzed by χ^2 analysis or Fisher's exact test, with statistical significance at $p < 0.05$.

Results

Fig. 1 shows the incidence of various FHR responses in relation to differing amounts of amniotic fluid. Twenty-nine patients (25.8%) were found to have oligohydramnios (amniotic fluid index ≤ 5.0 cm), 30 patients (26.8%) had borderline fluid volume (amniotic fluid index 5.1 to 8.0 cm), and 53 patients (47.3%) had normal fluid volume (amniotic fluid index > 8.0 cm). The range of amniotic fluid index values was from 0.0 cm to 27.5 cm. There was no significant difference in the incidence of FHR decelerations after a sound stim-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Women's Hospital, Los Angeles County/University of Southern California Medical Center, and the Department of Obstetrics and Gynecology, Tripler Army Medical Center.
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Reprint requests: Albert P. Sarno, Jr., MD, Major, Department of Obstetrics and Gynecology, Tripler Army Medical Center, Honolulu, HI 96859-5000.
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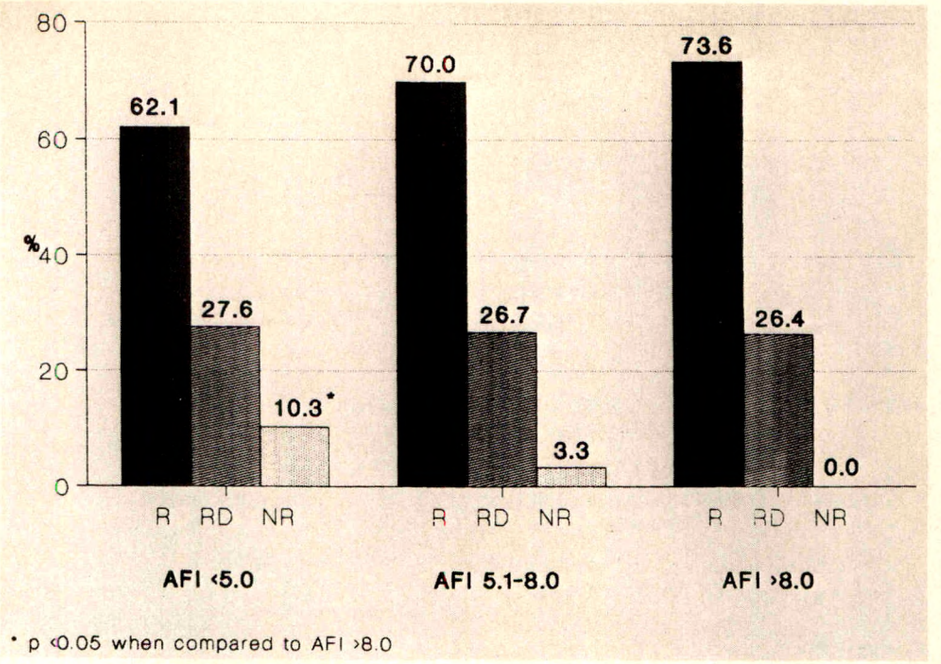


Fig. 1. FHR response to fetal acoustic stimulation grouped by amniotic fluid index. *R*, Reactive; *RD*, reactive FHR with deceleration; *NR*, nonreactive; *AFI*, amniotic fluid index in centimeters.

ulus in any subgroup for which amniotic fluid volume was determined. In the presence of oligohydramnios, however, significantly more patients exhibited nonreactive responses when compared with patients who had normal fluid volumes (10.3% vs 0.0%, $p = 0.04$).

Comment

Recently, Menashe and Sherer[2] recommended caution in the use of sound stimulation in cases of oligohydramnios because of the anecdotal experience that variable deceleration of the FHR was more common in this setting.

Therefore before acoustic stimulation is widely applied, the concern that is raised in this anecdotal report regarding oligohydramnios must be addressed.

This study addresses the role of amniotic fluid volume during acoustic stimulation. The findings suggest that amniotic fluid volume appears to play an insignificant role in the genesis of FHR decelerations that occur after fetal acoustic stimulation. Regardless of amniotic fluid volume, approximately 25% of patients exhibited variable decelerations. The vigorous fetal startle reflex that is associated with sound stimulation most likely results in transient cord compression that is independent of amniotic fluid volume. Therefore it does not appear necessary to withhold fetal acoustic stimulation in the presence of oligohydramnios.

In conclusion, amniotic fluid volume seems to play an insignificant role in the genesis of FHR decelerations that occur after fetal acoustic stimulation.

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Successful pregnancy in a patient with Hallermann-Streiff syndrome

Susan L. Hendrix, DO, and Harold J. Sauer, MD

Lansing and East Lansing, Michigan

Reproductive capacity has not been studied in patients with Hallermann-Streiff syndrome, but successful pregnancy in these patients is rare. A patient with the classic signs—plus a müllerian developmental anomaly, hypertension, glaucoma, and an extensive surgical history—was artificially inseminated, and a term gestation ensued. (Am J Obstet Gynecol 1991;164:1102-4.)

Key words: Hallermann-Streiff syndrome, uterine anomalies, pregnancy

The Hallermann-Streiff syndrome was first described in three cases, the first by Hallermann in 1948[1] and two more reported by Streiff in 1950.[2] The majority of reports appear in the ophthalmology literature.

The Hallermann-Streiff syndrome is thought to be an autosomal recessive disorder with developmental anomalies, mainly affecting the cranial and facial bones. The abnormalities most frequently seen include proportional small stature (dwarfism), craniofacial disproportion (bird facies), microphthalmia, congenital cataracts, abnormal dentition, hypotrichosis, and cutaneous atrophy of the face and scalp. There is no apparent difference in sex distribution for this syndrome and most affected patients are of normal intelligence. The literature contains scant information about the reproductive potential of affected patients, except that infertility in men is related to cryptorchidism.

Case report

A gravida 0 woman presented to the Edward W. Sparrow Hospital Outpatient Obstetrics and Gynecology Clinic for an annual examination. She expressed an intense longing for a child, and she asked about the possibility of artificial insemination with donor semen.

This patient's extensive medical history began with a diagnosis of Hallermann-Streiff syndrome in infancy. She underwent multiple tympanocenteses and at least 10 ophthalmologic procedures for treatment of glaucoma and cataracts; at presentation she was receiving pilocarpine eye drops for therapy of glaucoma. For therapy of long-standing hypertension, she took three twice-a-day medications: hydrochlorothiazide 50 mg, hydralazine hydrochloride 25 mg, and propranolol hydrochloride 50 mg. She had undergone mentoplasty at age 16 years and rhinoplasty at age 17 years.

The patient was considered to be of normal intelligence. Menarche occurred at age 14 years. She denied sexual activity and contraceptive use. She initially had



Fig. 1. Patient with Hallermann-Streiff syndrome wearing wig.

From Edward W. Sparrow Hospital and the Department of Obstetrics, Gynecology and Reproductive Biology, Michigan State University.
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Reprint requests: Susan L. Hendrix, DO, Michigan State University, College of Osteopathic Medicine, 411 West Fee Hall, East Lansing, MI 48824-1316.
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Fig. 2. A and **B,** Photographs of patient with Hallermann-Streiff syndrome showing alopecia and craniofacial characteristics.

31-day cycles and biphasic basal body temperature charts, with ovulation occurring at day 17 or 18.

Physical examination. At presentation, the patient weighed 90 pounds (41 kg) and was 4 feet 7 inches (140 cm) tall; her body stature was otherwise proportional. She has hypotrichosis for which she wears a wig (Fig. 1). Despite extensive surgery, she has obvious craniofacial disproportion. Optic examination revealed small ocular globes, narrowed palpebral fissures, and a roving nystagmus. The patient's ears are small, and the nose is small and pointed (Fig. 2, *A* and *B*).

Pelvic examination showed normal external genitalia, a narrowed introitus, and a nulliparous cervix. The uterus was anteverted and normal in size and shape; no adnexal masses were palpated. The remainder of her physical examination was unremarkable.

Diagnostic procedures and tests. Hysterosalpingography suggested a bicornuate or septate uterus with bilateral tubal patency. Follow-up intravenous urography demonstrated no abnormalities. Hysteroscopy and diagnostic laparoscopy with chromopertubation showed a subseptate uterus, left tubal patency, and failure of right tubal filling.

Clinical course. The patient was informed of the uterine anomaly and was told how it might affect her reproductive potential. She was told that, in spite of three pregnancies reported in two women with this syndrome, no previous association with müllerian anomaly had been reported and thus prognostication



Fig. 3. Phenotypically normal son of patient with Hallermann-Streiff syndrome.

was not possible. She was counseled that the inheritance was probably autosomal recessive, but that autosomal dominant transmission could not be excluded.

She conceived on her second clomiphene citrate cycle with donor insemination at the Michigan State University Clinical Center. The pregnancy was uncomplicated until term; three serial ultrasonograms showed appropriate interval growth and no apparent fetal anomalies. A fourth ultrasonogram at 38 weeks' gestation suggested a small-for-gestational age fetus. At term pelvis had a narrow interspinous diameter, and the presenting vertex fetus was ballottable. A primary low-transverse cesarean section was performed while the patient was under epidural anesthesia, and a normal, viable male infant was delivered. The child is 4 years old and is phenotypically normal (Fig. 3).

Comment

This is the first reported case of Hallermann-Streiff syndrome associated with a müllerian anomaly, the outcome of which was a successful pregnancy. Of 70 affected patients in the literature, only two women have had successful pregnancies reported. This case supports the potential reproductive capacity of patients with the syndrome and is thereby useful in counseling women with the disorder.

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Fetal vibratory acoustic stimulation in twin gestations with simultaneous fetal heart rate monitoring

David M. Sherer, MD, Jacques S. Abramowicz, MD, Mary Lou D'Amico, RN, Christine B. Caverly, RN, and James R. Woods, Jr., MD

Rochester, New York

Sixteen vibratory acoustic stimulations were performed in seven normal twin gestations with continuous simultaneous recordings of each fetal heart rate response. All stimulations led to immediate synchronous fetal heart rate accelerations in both fetuses. This is in contrast to coinciding, yet nonsynchronous, spontaneous fetal heart rate accelerations that occur naturally. This observation suggests that different mechanisms are responsible for spontaneous and evoked fetal movements in twin gestations. (Am J Obstet Gynecol 1991;164:1104-6.)

Key words: Vibratory acoustic stimulation, twin gestation

Fetal movements may be spontaneous, originating within the fetus itself, or evoked, caused by external stimuli to the fetal environment. Among stimuli known to evoke fetal responses are fetal auditory, vibratory, tactile, visual and taste input, and maternal glucose intake.[1] It has been postulated that coinciding fetal heart rate (FHR) accelerations in twin gestations may represent in utero tactile communication between the two fetuses, one twin being stimulated by movements of the other.[2] To further investigate this issue we performed a prospective study involving 16 vibratory acoustic stimulations of seven twin gestations with simultaneous monitoring of each separate FHR response.

From the Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Strong Memorial Hospital, The University of Rochester School of Medicine and Dentistry.
Received for publication November 30, 1990; accepted November 30, 1990.
Reprint requests: David M. Sherer, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Rochester School of Medicine and Dentistry, Strong Memorial Hospital, 601 Elmwood Ave., Box 668, Rochester, NY 14642.
6/1/27114

Material and methods

Between July 1, 1990, and November 1, 1990, seven twin gestations were studied. Simultaneous nonstress tests of fetuses from twin gestations were performed with the following technique: with the aid of a small portable real-time ultrasonographic unit (Aloka 210, Corometrics Medical Systems, Wallingford, Conn.) with a 3.0 MHz linear transducer, fetal positioning and the location of each fetal heart were determined.

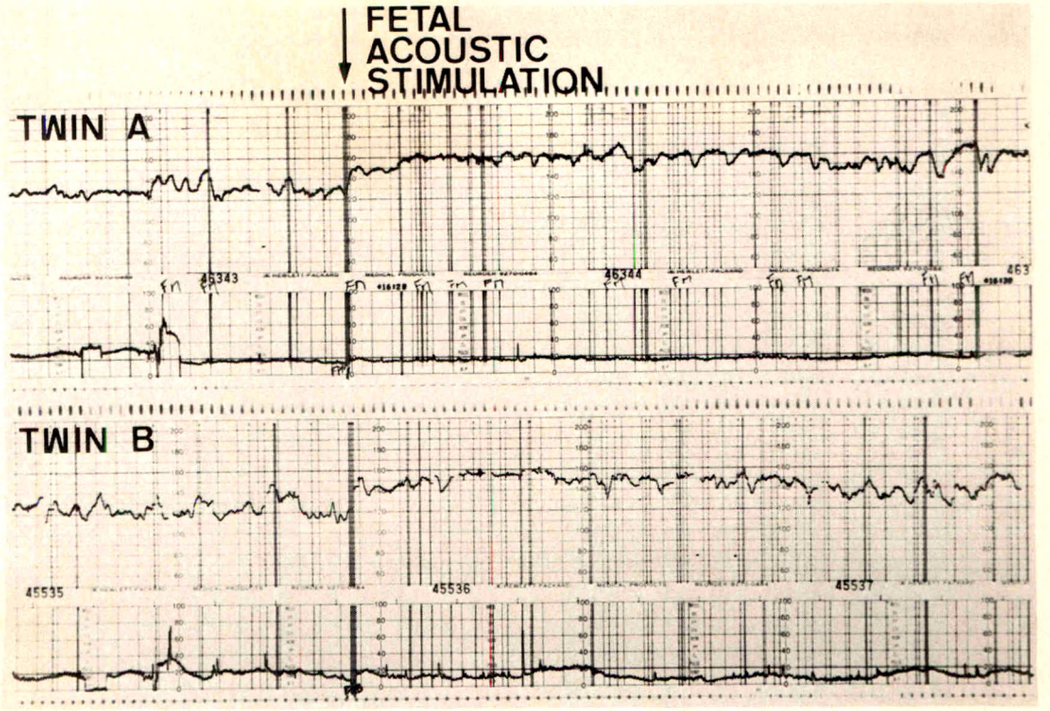


Fig. 1. Fetal vibratory acoustic stimulation of a twin gestation at 33 weeks with simultaneous electronic FHR monitoring with two separate cardiotocometers. Note the synchronous FHR accelerations of both twins in response to stimulus *(arrow)*.

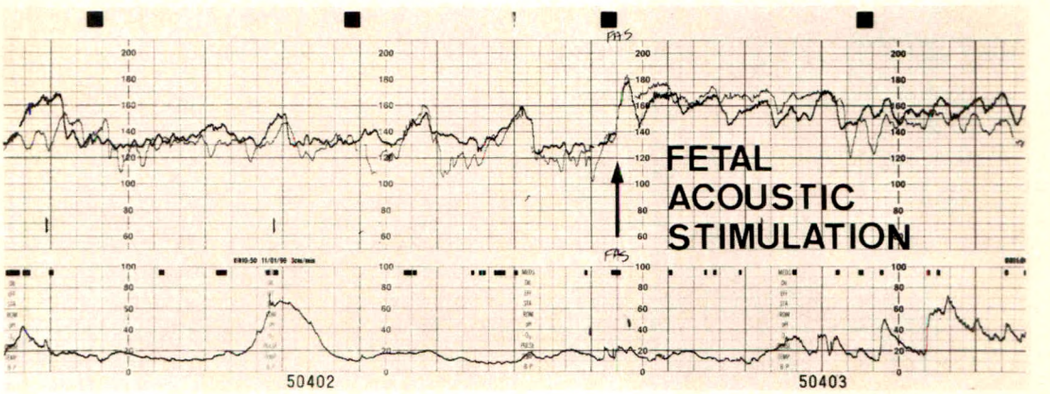


Fig. 2. Twin gestation at 37 weeks. Simultaneous FHR monitoring with a single cardiotocograph. Note two distinctly separate FHR recordings with synchronous FHR accelerations in response to stimulus *(arrow)*, as opposed to the four coinciding, yet nonsynchronous, FHR accelerations that occur spontaneously before the vibratory acoustic stimulation.

Two separate cardiotocograph machines (Hewlett-Packard Medical Products Group, Waltham, Mass., 8040A with identical ultrasonographic transducers; frequency = 1024 KHz, 15245A) or in the latter part of the study, a single cardiotocograph machine capable of performing simultaneous external FHR monitoring of twins (Hewlett-Packard M1350A with identical ultrasonographic transducers M1356A) were used. The paper speed was 3 cm/min. To assure that both fetuses were being monitored, nonidentical FHRs were verified before the stimulation. The tests were performed by a physician and a senior registered nurse. After a baseline FHR was established for each fetus, fetal vibratory acoustic stimulation was performed by applying an electronic artificial larynx (Model 5B, Western Electric, New York, N.Y., audible sounds = 750 to 1000 MHz,

vibrations between 110 and 200 Hz, output density 110 dB) for 5 seconds to the maternal abdomen directly above the maternal umbilicus.

Results

Mean maternal age was 24 ± 6 years, median gravidity was 1 (range, 1 to 3) and median parity was 0 (range, 0 to 2). Mean gestational age at the time of nonstress testing was 35 ± 2 weeks. The mean number of fetal acoustic stimulations performed on each patient was 2 ± 1 (range, 1 to 4). There was an interval of at least 1 week between stimulations in cases in which more than one vibratory acoustic stimulation per gestation was performed. Fetal presentations during the stimulations were: vertex vertex, six; vertex breech, six; breech vertex, two; breech breech, one; breech transverse, one. Mean gestational age at delivery was 36 ± 3 weeks. Mean birth weight of twin A was 2684 ± 440 gm and of twin B, 2654 ± 610 gm. Apgar scores of all 14 infants at 1 minute were ≥7.

Each of the described stimulations resulted in an immediate and synchronous acceleration of the FHR of both fetuses, irrespective of gestational age or fetal positioning (Figs. 1 and 2).

Comment

Previous studies of simultaneously recorded nonstress tests of twin gestations have revealed an association between the two separate FHR recordings. In a study of 50 twin gestations with a total of 152 nonstress tests, 57% of FHR accelerations were noted to coincide, occurring within 15 seconds of each other.[2] It was thought that this rate of coinciding FHR accelerations could be explained by the first twin's spontaneous movements causing vibration evoking a tactile response of the second twin, thereby producing fetal movements with associated FHR acceleration of the second twin. In that study, only 9% of spontaneously coinciding FHR accelerations were precisely synchronous.[2] Sadovsky,[1] in a study of 10 twin gestations with simultaneous ultrasonography of each twin, noted a 24.3% rate of simultaneous fetal movements and postulated that these represent evoked fetal movements in response to external stimuli, as opposed to the 75% of spontaneous (not simultaneous) fetal movements. Fetal acoustic stimulation, when applied to nine sets of twins, caused vigorous simultaneous fetal movements of both twins observed by ultrasonography.[1] Our documentation of the immediate synchronous FHR accelerations of both twins in response to similar stimulation supports by a different method Sadovsky's observations and suggests that different mechanisms are responsible for spontaneous and evoked fetal movements in twin gestations.

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Fetal death associated with measles virus infection of the placenta

Kazuko Moroi, MD,[a] Siu Saito, MD,[b] Takeshi Kurata, MD, PhD,[c] Tetsutaro Sata, MD.[c] and Masahiko Yanagida, MD[a]

Tokyo, Japan, and Bethesda, Maryland

A case of fetal death at 25 weeks' gestation, which was associated with maternal measles infection, is described. Immunohistochemical study revealed measles virus antigen in the syncytial trophoblastic cells and decidua but not in the fetus. Results suggest that fetal death was caused by measles virus infection in the placenta. As far as we know, this is the first report in which a measles virus antigen was detected in the placenta by the immunohistochemical method. (AM J OBSTET GYNECOL 1991;164:1107-8.)

Key words: Maternal measles, placental infection, fetal death

Measles virus infection during pregnancy is rare and usually does not result in stillbirth. Thus little is known about fetal death that is caused by measles virus infection in the mother. A significant association was demonstrated between maternal measles and prematurity but not between maternal measles and spontaneous abortion.[1]

We report on a stillbirth that occurred at 25 weeks' gestation, probably because of measles virus infection of the placenta.

Case report

A 28-year-old woman, gravida 1, para 1, was referred to our hospital at the 23 weeks' gestation because of threatened abortion that was associated with high fever and skin eruptions that had continued for 3 days. Family and medical history were unremarkable, and the current pregnancy was uneventful.

At the time of admission, the patient had a fever of 40° C. Pulse rate was 130 beats/min and blood pressure was 112/60 mm Hg. Physical examination revealed dyspnea and eruptions on the face, body and thighs. The uterine fundus was consistent with 23 weeks' gestation. The cervix was closed, 60% effaced, and posterior. The slight leakage of amniotic fluid was clear. Ordinary bacterial culture of vaginal discharge was negative. Ultrasonographic examination revealed a normal-size fetus without abnormal echo and with a normal amount of amniotic fluid. The fetal heartbeat was 180 to 190 beats/min, and its pattern was reactive. Routine blood studies and kidney and liver function studies were within normal limits. Proteinuria, ketonuria, and bacteriuria were observed. Antibody titer to measles virus, which was determined by complement fixation test, changed from negative, at the time of admission, to positive (×16), on the ninth day of her illness.

The patient complained of fever, conjunctivitis, and a sore throat. She had blisters on the tongue and respiratory difficulties that were accompanied by a heart murmur. On day 9 of her illness these signs disappeared rapidly. On day 13, when she was free from almost all signs of the disease, the fetus was suddenly expelled. The stillborn infant was 650 gm, male, and normal in body shape.

The placenta was 180 gm, oval-shaped, and hard in consistency. The fetal side of the placenta was dark and the maternal side was pale and yellowish, which suggests deciduitis. Thrombosis was not found macroscopically on the cut surface of the placenta. Histologic examination revealed intervillous diffuse infiltrations of fibrin and mononuclear cells. Adhesions of the villi that were accompanied by necrosis of the trophoblastic cells were observed. The umbilical cord appeared edematous without inflammatory changes.

Measles virus antigen was demonstrated in the decidua of the placenta by immunofluorescent and immunoperoxidase methods.[2] In syncytial trophoblastic cells, the virus antigen was also clearly observed (Fig. 1). Neither inflammatory change nor measles virus antigen was found in any organs of the fetus.

Comment

As far as we know, there have been no reports that suggest an association between maternal measles and placental infection that leads to spontaneous abortion. This may be the first case of measles virus infection of the placenta that resulted in fetal death.

Placental transmission of measles virus does not seem to occur frequently.[1] In this case there was no evidence of measles virus infection in the fetus, in spite of diffuse infection of the syncytial trophoblastic cells and decidua.

From the Department of Obstetrics and Gynecology, Tokyo Metropolitan Tsukiji Maternity Hospital,[a] the Department of Clinical Pathology, Juntendo University Medical Hospital,[b] and the Department of Pathology, National Institutes of Health.[c]
Received for publication July 26, 1990; revised October 29, 1990; accepted November 28, 1990.
Reprint requests: Kazuko Moroi, MD, Tokyo Metropolitan Tsukiji Maternity Hospital, Akashi-cho 6-24, Chuo-ku, Tokyo, Japan.
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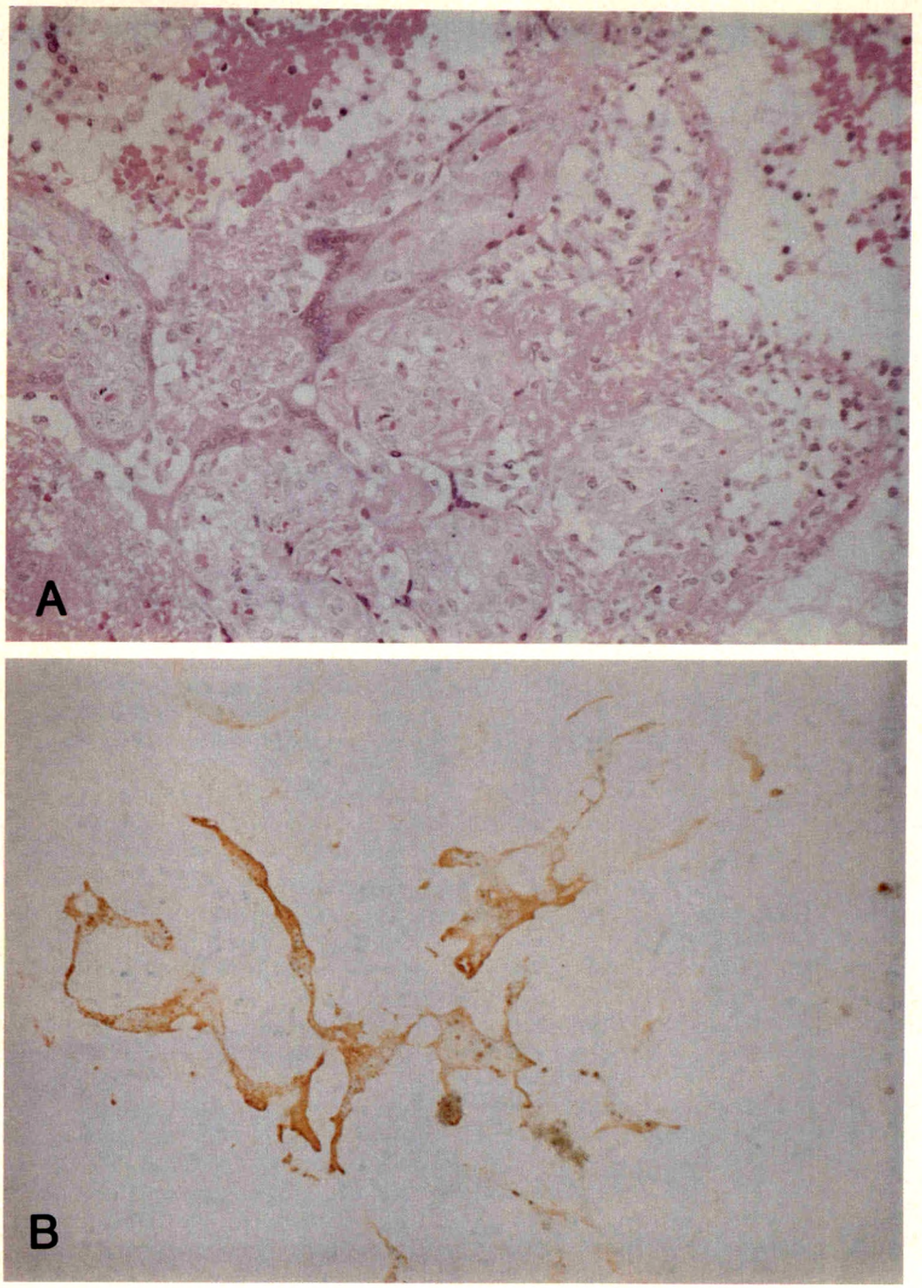


Fig. 1. Histopathologic lesion and measles virus antigen in placenta. **A**, Multinucleated cells and exudation of fibrin around chorionic villi. (Hematoxylin-eosin.) **B**, measles virus antigen in syncitial trophoblastic cells. (Immunoperoxidase.)

We suggest that fetal death was not primarily caused by fetal infection of measles virus but rather by severe placental damage, including fibrin infiltration of the perivascular and intervillous spaces of the placenta. These changes may decrease intravillous blood flow and oxygen supply to the fetus, which cause fetal death or distress that probably produces uterine contractions.

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Coffee during pregnancy: A reproductive hazard?

Steven A. Narod, MD, Silvia de Sanjosé, MD, PhD, and Cesar Victora, MD, PhD
Lyon, France, and Pelotas, Brazil

Coffee is consumed in large quantities worldwide and any adverse effects would likely have important public health consequences. Because of the widespread exposure to coffee and other caffeine-containing beverages and because teratogenic effects of caffeine have been recorded in several species since 1960, women are concerned that there may be reason to limit their intake of coffee when pregnant. Several human studies on birth defects have been conducted and the overall results do not implicate coffee as a likely human teratogen. However, there is some evidence that consumption of three or more cups of coffee per day may have a modest effect on lowering infant birth weight. Studies of coffee consumption and increased rates of spontaneous abortion and delayed time to conception are inconsistent and conclusions cannot yet be drawn. (AM J OBSTET GYNECOL 1991;164:1109-14.)

Key words: Coffee, caffeine, congenital malformation, spontaneous abortion, birth weight, infertility

Coffee has international social and medical acceptance and is a leading commodity of world trade. In the United States consumption is estimated at 4.5 kg per person per year, or 1.7 cups per day (an average of 3.3 cups per coffee drinker).[1] However, the highest consumption of coffee is recorded in Finland, with 12.2 kg per person per year. Many women now decrease their coffee intake when pregnant,[2] possibly in response to several publicized reports of adverse reproductive effects including congenital malformations, decreased birth weight, spontaneous abortion, and reduced fertility. Recently a meeting was conducted at the International Agency for Research on Cancer in Lyon, France, to evaluate the carcinogenic risks of caffeine-containing beverages. The epidemiologic and laboratory-based evidence for adverse effects of coffee on several aspects of human health, including reproduction, was reviewed.[5]

Caffeine is the predominant pharmacologically active component of coffee and is responsible for its stimulating effect. Caffeine is also used to stimulate spermatozoa in vitro with the hope of increasing the conception rate in artificial insemination.[4] The concentration of caffeine in coffee varies according to the method of preparation, making international epidemiologic studies difficult. In North America a typical cup of brewed or percolated coffee contains roughly 100 mg of caffeine. Other caffeine-containing beverages include tea (40 mg per cup) and most cola beverages (20 mg per 10-ounce serving).

Caffeine was shown to be a teratogen in mice in 1960.[5] Since that report, adverse developmental effects of caffeine were confirmed in several species including mice, rats, and rabbits; these effects were recently reviewed[6] and are not discussed here in detail. Mice are susceptible to cleft palate and abnormalities of the digits including ectrodactyly (absence of fingers) at doses of caffeine >50 mg/kg per day (equivalent to about 25 cups of coffee per day or roughly two to five times higher than the highest consumption levels regularly reported by pregnant women). Ectrodactyly appeared in rabbits at doses >100 mg/kg per day.[7] In most teratology studies effects were seen only at >50 mg/kg per day (i.e., at doses in which maternal toxicity was common).

The reports of defects in mice induced by caffeine naturally raised concern about the possibility of similar effects in human beings. In 1980, on the basis of the animal evidence, the U. S. Food and Drug Administration cautioned pregnant women to limit their intake of coffee.[8] A considerable amount of epidemiologic data were collected since then and we are now in a better position to use information about human exposures to judge the need for such a warning.

Congenital malformations

In 1981 three cases of children with ectrodactyly were born to women who admitted to drinking large

From the Unit of Mechanisms of Carcinogenesis and the Unit of Field and Intervention Studies, International Agency for Research on Cancer, and the Department of Social Medicine, University of Pelotas. Reprint requests: Silvia de Sanjosé, MD, PhD, International Agency for Research on Cancer, 150 Cours Albert Thomas, Lyon, France 69372.
6/1/25240

amounts of coffee during pregnancy.[9] Some limb malformations may be relatively minor, but ectrodactyly is clearly a serious defect; in some a "lobster-claw" or "split-hand" malformation is present. Ectrodactyly may be seen in combination with a cleft palate and may appear in several members of a family; however, the majority of cases occur sporadically and have no known cause.[10]

Three large-scale epidemiologic studies[11-13] published in 1982 and in 1983 failed to support this anecdotal finding. In Finland, coffee was drunk in greater quantities during pregnancy by mothers of children born with congenital malformations, when compared with local control mothers.[11] These negative results are consistent with those of most other studies. Linn et al.[12] interviewed 12,205 pregnant women in the Boston area from 1 to 2 days after delivery. Major malformations were documented in 2.5% of the women who reported no coffee or tea use during pregnancy and in 2.0% of heavy coffee drinkers. In the third study[13] mothers of 2030 malformed infants in Boston, Philadelphia, and Toronto were also questioned about coffee drinking in pregnancy. The level of consumption did not vary significantly for children with malformations of different organ systems.

More recently, Tikkanen and Heinonen[14] reported on coffee consumption in the mothers of 569 children born with cardiovascular malformations in Finland between 1982 and 1984 and in 1052 control mothers. Eighty-two percent of mothers in both the case and control groups reported drinking coffee during pregnancy.

The positive evidence to date for a possible teratogenic effect of coffee in human beings comes from two less rigorous studies. In an early Belgian case-control study, the mothers of 190 children with birth defects drank an average of 28 cups of coffee per week during pregnancy, three cups more than the 175 control mothers.[15] However, 44 mothers in the case group (23.2%) drank more than 8 cups per day versus 21 mothers in the control group (12.9%; $p < 0.05$). The odds ratios for heavy coffee drinkers can be calculated for several malformations including ventricular septal defect [odds ratio (OR), 23.5], cleft lip (OR, 4.5), hypospadias (OR, 4.8), Down syndrome (OR, 3.0), and limb defects (OR, 1.7).

The second positive study is from Japan[16]; 9921 healthy pregnant women were asked about coffee consumption at 24 weeks' gestation. The rate of congenital anomalies in coffee drinkers (3.7%) was more than twice as great as the rate in nondrinkers (1.7%). Surprisingly, the authors fail to comment on this highly significant ($p < 0.001$) difference, and state instead, "The rate of congenital anomalies was not significantly different among the five groups."[16] Elevated rates were seen for cardiac malformations [relative risk (RR), 1.9], cleft palate (RR, 2.4), polydactyly (RR, 2.9), multiple malformations (RR, 21.5), chromosomal abnormalities (RR, 14.0), and miscellaneous anomalies (RR, 1.8). Many of the details that the reader would like to see are missing from this provocative report. It is noteworthy that in both positive studies malformations as a result of chromosomal abnormalities were increased among children of heavy coffee drinkers. If the alleged association with coffee were causal, then the beverage would qualify both as mutagen and teratogen—a simpler explanation is that a hidden bias is elevating rates of malformations of all types.

Although ectrodactyly was not specifically analyzed in the three high-quality reports (probably because of the rarity of the condition), positive associations with skeletal anomalies as a group were not found. However, very few women were exposed regularly to large amounts of coffee (e.g., eight or more cups per day), and because of the possibility of a threshold level for teratogenic effects in human beings, the safety of coffee consumption at high levels cannot be inferred from studies of predominantly low or moderate coffee drinkers.

Incidence data on specific congenital anomalies for 10 European countries are compiled and published by the EUROCAT project.[17] There appears to be little correlation between the frequency of abnormalities of the limbs and average coffee consumption (as determined by the International Coffee Organization[1]) among the 17 contributing registries. Correlation coefficients obtained by combining data from these two sources can be calculated for coffee consumption and limb reduction defects (−0.065), syndactyly (0.435), polydactyly (0.201), and all limb deformities combined (0.0122). Denmark, with the highest per capita coffee consumption recorded for any of the participating countries (10.83 kg per person per year), had the highest rate of all limb anomalies (possibly a reflection of the high rates of reporting in the country).

In summary, at levels commonly consumed during pregnancy, epidemiologic studies do not support the animal data that coffee may be a human teratogen. The findings of the two discrepant studies deserve mention although their data should probably be reexamined at this time.

Birth weight

Low birth weight (defined here as <2500 gm) is the most extensively studied reproductive effect in relation to coffee. Infant birth weight is easy to measure, is measured routinely, and is a meaningful measure because it correlates with infant mortality and morbidity. In developed countries, the majority of low-birth-weight babies are preterm, but other causes are re-

Table I. Summary of studies of coffee and birth weight

Study	*Risk study population*	*Principal factors adjusted for*	*Results*
Watkinson, Fried[3]	286	Smoking, education	Decreased birth weight among consumers of >300 mg per day*
Linn[12]	12,205	Alcohol, smoking	<4 cup/day, RR = 1.0 ≥4 cup/day, RR = 1.2†
Furuhashi[16]	9,921	None	<5 cup/day, BW = 3163 gm >5 cup/day, BW = 3081 gm*
Mau, Netter[19]	5,200	Alcohol, maternal age	Nondrinkers, RR = 1.0 Occasional, RR = 1.4 Frequent, RR = 1.6*
VandenBerg[20]	15,000	Smoking, reproductive history	≤1 cup/day, RR = 1.0 2-6 cups/day, RR = 1.4 ≥7 cups/day, RR = 2.2*
Kuzma, Sokol[21]	5,093	Gestational age, weight gain, ethnic group, smoking	Decreasing birth weight with increasing coffee intake*
Martin, Bracken[22]	3,891	Gestational age, ethnic group, parity, smoking, alcohol	No caffeine, RR = 1 1-150 mg, RR = 1.4 151-300 mg, RR = 2.3 >300 mg, RR = 4.6*
Munoz[23]	48	Smoking, alcohol, gestational age	Average decrease of birth weight of 121 gm for drinkers of >450 ml/day vs none
Caan, Goldhaber[24]	267	Ethnic group, alcohol, parity, smoking, weight	Nondrinkers, RR = 1.0 >300 mg/day, RR = 2.8†
Brooke[25]	1513	Gestational age, height, parity, smoking, sex of baby	0-200 mg, BW = 3664 gm 200-400, BW = 3609 mg† >400, BW = 3556 gm†

BW, Birth weight.
*$p < 0.05$.
†Not significant.

sponsible for about one third. The study of low birth weight is complicated by the many demographic, medical, social, and behavioral characteristics that influence it, and coffee consumption has been associated with several of these. In particular, smoking, a known risk factor for low birth weight, may be positively correlated with coffee intake.[12] Not all studies have considered these important relationships. It should be noted that the probability of a low-birth-weight baby having a more serious developmental outcome varies with the cause of the low birth weight. Children small for dates born to smoking mothers, for example, may be less likely to have long-term growth problems than babies born with a similar birth weight as a result of other causes.[18]

Current evidence of an association between coffee (and other caffeine-containing beverages) and low birth weight is summarized in Table I. Smoking was controlled for in eight of the 10 reports. An inverse relationship between coffee consumption and birth weight was seen in some degree in all studies, and on seven occasions the association was statistically significant. Relative risks of being delivered of an infant who weighs <2500 gm ranged from 4.6 to 1.2 for drinkers of more than tree cups of coffee per day. The mean decrease in birth weight in the positive studies ranged from 70 to 121 gm—values slightly lower than those reported as a result of smoking (roughly a 150 to 200 mg reduction).[26]

Among these reports three studies have not been reviewed elsewhere. The most relevant is a comprehensive study from New Haven, Connecticut,[22] of 3891 women who attended selected antenatal care centers. Total caffeine consumption was estimated for these women from reported intake of coffee, tea, and cola drinks at home interviews during pregnancy. Pregnancy outcomes were determined from hospital records. After controlling for age, ethnic group, parity, and smoking, caffeine intake >300 mg per day (equivalent to three cups of coffee a day) was associated with a 4.6-fold increase in low birth weight in comparison with nondrinkers [95% confidence interval (CI) 2.0 to 10.5]. A dose-dependent response with increasing consumption was seen. Coffee intake was also associated with low socioeconomic status, smoking, and alcohol intake. The effect of caffeine on birth weight was limited to term infants.

In a case-control study in California[24] low-birth-weight babies were more common to mothers who drank more than three cups of coffee daily during the

first trimester. After adjusting for ethnicity, alcohol, smoking, prepregnancy weight, weight gain, and parity the risk was elevated (RR, 2.8) but was not statistically significant (95% CI, 0.89 to 8.69).

Brooke et at.[25] studied 1513 pregnant women in London. After correcting for gestational age, maternal height, parity, sex of the baby, and smoking they found no relationship between the mean birth weight and coffee consumption.

Earlier studies of coffee and infant birth weight were reviewed elsewhere.[27-28] Overall, the data on coffee consumption during pregnancy suggest a small effect on reduction of birth weight. This effect is most likely to be relevant for women who are already at risk of having a low-birth-weight infant by virtue of other medical and social factors.

Spontaneous abortions

An association between coffee consumption and spontaneous abortion was found in three studies reviewed,[16, 29, 30] but not in three others.[2, 31, 32] Srisuphan and Bracken[29] interviewed 3135 women during their pregnancies (for methods see reference 22). They found a 70% increase in miscarriage at between 8 and 26 weeks' gestation in moderate coffee drinkers compared with nondrinkers after correcting for differences in maternal age, gestational age, previous spontaneous abortions, and other gynecologic surgery ($p = 0.03$).

Furuhashi et al.[16] reported an increase in the rate of spontaneous abortions from 1.2% to 2.0% for mothers who drank coffee during their pregnancies ($p < 0.001$). The methodologic limitations encountered in this study were mentioned previously.

The study by Weathersbee et al.[30] is much cited, no doubt because of its dramatic results. Eight hundred obstetric patients were identified in Utah and southern Idaho in 1974 and 1975, of whom 489 completed a mailed questionnaire. Sixteen women reported coffee intake >600 mg daily. Eight of the 16 pregnancies ended in miscarriage, five in stillbirth, and two in premature births. Unfortunately, the authors sampled from a group of women hospitalized because of a variety of obstetric reasons, and the rates reported are difficult to interpret. A more meaningful analysis would have been to compare coffee levels among samples of women with miscarriages and other reasons with control patients who had normal deliveries. In some cases, women were asked to complete the questionnaire 2 years after pregnancy of interest.

Watkinson and Fried[3] found no differences in the mean caffeine intake between women who had miscarriages and those with normal deliveries, although a higher proportion of coffee drinkers were found among mothers who miscarried. Krsnjavi and Mimica[31] in Yugoslavia and Ardanova and Katsulov[32] in Bulgaria reported no relationship between the rate of spontaneous abortions and coffee drinking. In most studies pregnant women have been recruited from prenatal clinics and therefore any effects of coffee on very early miscarriage will be missed.

Fertility

Recently, attention has been drawn to a possible effect coffee may have on delaying fertility.[33-36] A series of 124 women who had not become pregnant by 3 months after discontinuation of birth control were questioned with regard to habitual coffee consumption and were then monitored for the next 12 menstrual cycles or until they became pregnant.[33] A total of 452 cycles were analyzed; with a life-table approach the authors calculate that 28% of high coffee consumers (>31.5 cups of coffee per month) were unable to achieve pregnancy in the first year versus 6% for women who consumed less than this amount. The probability of becoming pregnant in a given month in the group with high coffee consumption ranged from 32% to 88% of that of the more moderate drinkers. A second study[34] with a much larger number of women (2817) was unable to confirm the alleged relationship between coffee consumption and conception. The average reported time to conception was 4.4 months for women who drank three or more cups per day versus 4.6 months for those who drank less than one cup per day. The overall patterns of fertility were similar in the two centers where the studies were performed and there is no apparent reason why the results of the two studies should be so discrepant. In the original study the amount of coffee consumed was recorded before conception, whereas in the second study consumption levels were determined retrospectively. However, because of the larger number of participants in the second study it should probably be given greater weight. The findings of Wilcox et al.[33] prompted a second look at a data set that was generated from the interviews of more than 6000 pregnant women before 1967.[35] Women who reported having had trouble getting pregnant were almost twice as likely to be high consumers of coffee (four or more cups per day) than were those without this difficulty, but significance figures are not provided. In a similar manner the records of 3010 married women living in Massachusetts who were delivered of a single infant between 1977 and 1980 were reviewed.[36] Mean time to conception was longer for women who drank four or more cups of coffee a day (6.65 months) than for women who did not drink coffee (4.84 months). For women who consumed four or more cups of coffee a day average fecundability (calculated monthly) was 81% of that of those who did not drink coffee, after adjusting for age, parity, weight, alcohol, tobacco, and marijuana use ($p < 0.05$). These four studies raise an

interesting hypothesis, but an association is yet to be proved. It is hoped that further data will be available soon.

Comment

The epidemiologic data assembled to date on congenital malformations and coffee consumption during pregnancy do not confirm the teratogenic associations observed in mice. To date associations in human beings are largely anecdotal, although it is recognized that little data are available on consumers of very high amounts of coffee during pregnancy, that a threshold effect is possible, and that if a teratogenic effect were limited to very rare malformations (e.g., ectrodactyly) these may be missed in studies of birth defects as a group. The studies reviewed vary in quality. In general it was not possible to verify the amount of coffee reported to be consumed by the pregnant women and in some cases women were asked to recall habits of several years past. It may be that the women with poor outcomes were more likely to overestimate these amounts.

Although evidence is limited, coffee drinkers may be at higher risk of having a baby of low birth-weight. On the basis of the data it is probably wise to reduce coffee consumption during pregnancy to a moderate level, for example not more than 2 cups of percolated coffee per day, especially for women who are at risk of a low-birth-weight infant because of other reasons. Because fetal growth is most rapid during the third trimester this may be the period of greatest sensitivity, but the studies conducted so far have not tested this hypothesis specifically. Three of four studies on fertility reported delayed time to conception among women who drank moderately large amounts of coffee. Although further evidence is needed to confirm this association, it is probably reasonable to suggest to women who have difficulty in becoming pregnant that they limit their coffee intake. The evidence is so far insufficient to support an association between coffee drinking and spontaneous abortion.

In summary, the need for a warning for pregnant women to limit their coffee drinking is probably justified, although amounts normally consumed by pregnant women in the United States likely are safe. It is not clear whether the effect on birth weight is due entirely to caffeine or to other components of coffee. Because of the increasing use of decaffeinated beverages this may be a question for future research.

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A new model for objective assessment of cervical ripening: The effect of prostaglandin E_2 and prelabor contractility

Toru Takahashi, MD,[a] Beth Marcus, BA,[b] Richard G. Scheerer, MD,[b] and Michael Katz, MD[b, c]

Kawasaki, Japan, and San Francisco, California

A rabbit cervix preparation designed to evaluate the effects of locally administered substances and those of prelabor contractility on cervical ripening is described. In one experiment, one of the two cervices was exposed to prostaglandin E_2 gel and the contralateral cervix was exposed to plain gel. In another experiment, one cervix was completely isolated from prelabor contractility, whereas the contralateral cervix remained intact. Cervical ripening was assessed by measurement of the stretch modulus and rate of creep 2 days later. Treatment with prostaglandin E_2 gel and exposure to prelabor contractility resulted in significant unilateral cervical changes, suggesting that at least part of the prostaglandin E_2 effect is locally mediated and that spontaneous prelabor contractility may play a role in cervical preparation for labor. Furthermore, because of its simplicity, this animal preparation can be useful in the investigation of the effects of other locally administered substances on cervical ripening. (AM J OBSTET GYNECOL 1991; 164:1115-8.)

Key words: Cervical ripening, rabbit, prelabor contractility, prostaglandin E_2, local effect

The effect of locally applied hormones on ripening of the uterine cervix has been well documented by numerous investigators.[1-4] However, it remains unclear whether the effect occurs directly through local diffusion or indirectly by triggering events in sites remote from the cervix, which eventually may result in cervical ripening. For example, vaginal prostaglandin application may initiate subtle myometrial activity[5] that in turn affects cervical status. To distinguish direct from indirect effects, we designed an animal model that permits two cervices to undergo different manipulations while under the influence of the same systemic environment. In this article we describe our initial experiments with this new animal preparation, which was designed to evaluate the effects of local manipulations on cervical ripening. The manipulations in this study include the local application of prostaglandin E_2 (PGE_2) and the isolation of one cervix from prelabor uterine contractility.

From the Department of Obstetrics and Gynecology, Nippon Medical School,[a] Children's Hospital,[b] and the University of California, San Francisco.[c]
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Reprint requests: Michael Katz, MD, Department of Obstetrics and Gynecology, M-1489, University of California, San Francisco, San Francisco, CA 94143.
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Material and methods

Ten New Zealand White rabbits, weighing 4 to 5 kg and with known dates of conception, were used. Guidelines for animal use were approved by the Research and Human Experimentation Committee and the Animal Care Subcommittee of our institution. We studied only animals in which the number of fetuses in each horn was similar (difference of two or fewer). On 27 to 28 days' gestation (term is 31 days), the uterine horns were exposed through a lower abdominal midline incision after induction of general anesthesia with ketamine (20 mg/kg per hour) and xylazine (4 mg/kg per hour). Induction of local anesthesia with 1% lidocaine was used for skin and peritoneal incisions. The two cervices and the proximal half of the vagina were separated by use of a surgical stapler (TA 55 surgical stapling instrument, United States Surgical Corporation, Norwalk, Conn.) as shown in Fig. 1. Preliminary studies with the use of this preparation on a few animals showed that the surgery did not interfere with the animals' capability of spontaneous delivery at term. Experiments were designed to evaluate the effects of either local PGE_2 application (group 1, $n = 5$) or prelabor uterine contractility (group 2, $n = 5$) on cervical ripening. In group 1, 1 ml of gel containing 0.25 mg of PGE_2 was applied to the portio vaginalis cervicis of one side and 1 ml of plain gel was applied to the other. In group 2 the uterine horn on one side was separated from the cervix so that prelabor contractions descend-

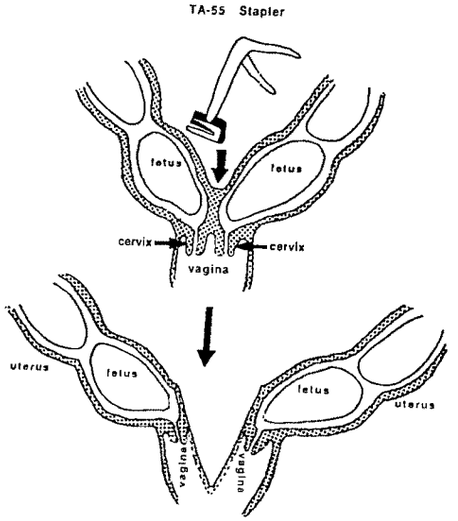


Fig. 1. Schematic presentation of two cervices and the proximal half of the vagina separated by use of a surgical stapling instrument.

ing from that side would not reach the cervix (Fig. 2). The manipulations were alternated between left and right sides in successive experiments. A third set of experiments was done to determine cervical distensibility in the nonpregnant rabbit in early pregnancy and post partum. In these animals, no gel was applied to the cervix.

Two days later (day 29 to 30) the animals were anesthetized with pentobarbital (30 mg/kg) and laparotomy was performed. After confirmation of fetal viability the cervices were removed and cut transversely into several 1.5 mm wide rings. Two central rings from each cervix were randomly assigned to one of two tests of tensile properties. One method, which was initially described by Harkness and Harkness[6] and then modified by Hollingsworth and Isherwood,[7] applies a constant load to the cervix and measures the increments in length plotted against time. This method (the creep test) defines cervical extensibility as equal to $10^3K/L_o$, where K is the rate of increase in inner circumference during the slow deformation (millimeters per minute) and L_o is the inner circumference in millimeters at time zero. This formula provides a measure of the rate of increase in cervical diameter corrected for variations in cervical size.

The second method we used to evaluate cervical ripening was the stretch modulus in which cervical distensibility is determined by continuous measurement of the tension that is created while stretching the specimen at a constant rate.[8] Cervical ring stretch modulus is calculated from the linear part of the time-tension curve with the formula $E = (T/A)/(L/L_o)$, where E is the stretch modulus in milligrams per square millimeter, A is the cross section of the analyzed sample in square millimeters, T is the recorded tension in milligrams, L is the length increment in millimeters, and L_o is the unstressed initial length in millimeters.

Means and SDs were calculated for each of the two methods of cervical assessment, and comparisons between the control and experimental sides were done by the Wilcoxon paired-sample test.

Results

The differences in the rate of creep and stretch modulus between the control and experimental sides for the PGE_2 studies confirmed the presence of a significant effect. The mean rate of creep for the PGE_2-treated side was 3.47 ± 1.41 min^{-1} as compared with 2.01 ± 1.17 min^{-1} for controls ($p < 0.05$). The mean stretch modulus for the PGE_2-treated side was 4250 ± 1360 mg/mm^2 as compared with 7580 ± 3560 mg/mm^2 ($p < 0.05$) for controls. Rate of creep was higher and stretch modulus was lower for the PGE_2-treated cervices of each animal.

In the second group of experiments in which one cervix was separated from the uterine body, the mean rate of creep for the control intact cervices (exposed to prelabor contractions) was 4.23 ± 2.25 min^{-1} as compared with 2.98 ± 1.58 min^{-1} for the separated cervices (not exposed to prelabor contractions, $p < 0.05$). The mean stretch modulus for control intact cervices (6090 ± 3490 mg/mm^2) was significantly lower than that observed for the separated cervices (8760 ± 2990 mg/mm^2). The higher rate of creep and lower stretch modulus values were observed in the intact cervix in each of the experiments.

For comparison, in the nonpregnant rabbit, rate of creep was 1.05 ± 0.27 and increased to 1.66 ± 0.22 in early pregnancy (7 days' gestation), increased further to 5.05 ± 1.00 at 29 to 30 days' gestation, then fell to 1.91 ± 0.60 at 7 days post partum. Similarly, stretch modulus was 5890 ± 1640 in nonpregnant rabbits, fell to 4040 in early pregnancy, was 3280 ± 1450 at 29 to 30 days' gestation, and was 3240 ± 1380 at 7 days post partum.

Comment

Our data for control cervices show that even with timed pregnancies and vigorously controlled conditions there are significant individual variations in cervical characteristics among animals. This fact stresses the potential difficulties in comparison of the effects of drugs or uterine contractility on the cervix between animals and points to the potential advantage of our preparation, which uses each animal as its own control.

In this study we were interested in the way PGE_2 affects cervical ripening. Mackenzie and Embrey[9] showed by use of tocodynamometry that cervical ap-

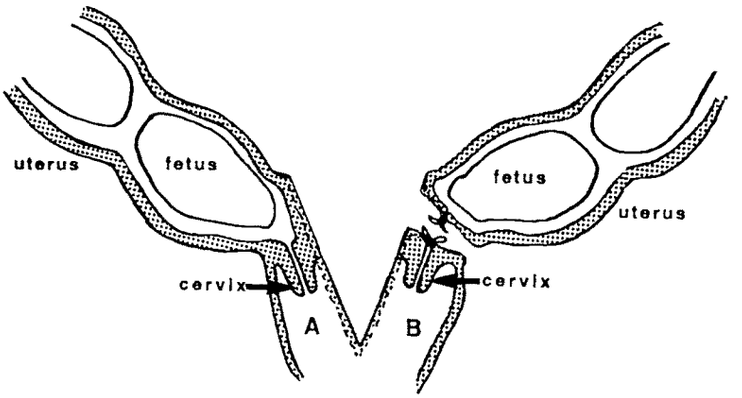


Fig. 2. Uterine horn on one side is separated from the cervix so that the cervix is not exposed to prelabor contractility descending from that side.

plication of PGE_2 in human beings can be associated with quick systemic absorption of the hormone, leading to increased uterine contractility. However, what proportion, if any, of the PGE_2-induced ripening can be attributed to a local effect or an increase in contractility is not clear. If PGE_2 were to initiate cervical ripening only through contractions, then one would expect that both cervices would be equally affected in our preparation. However, the significant differences in cervical ripening that we observed confirmed that at least part of the PGE_2 effect is local.

A second aspect of cervical ripening is that of a possible effect brought about by prelabor uterine activity. Stys[10] recorded intracervical pressures in ewes at term and concluded that cervical ripening preceded the onset of that uterine activity, which was characteristic of labor, by 12 hours. Liggins et al.[11] infused PGE_2 into the uterine artery of sheep and reported cervical ripening with relatively little uterine activity. However, until now there were no animal models available to evaluate whether the relatively low amplitude prelabor uterine activity plays a role in cervical ripening. Furthermore, even if uterine pressure is recorded, cervical changes depend to a significant extent on a variety of systemic hormonal changes that occur just before the onset of labor.[12] In this study, in which the systemic environment was identical for both cervices, exposure to prelabor uterine activity was shown to be associated with a significantly more compliant cervix than that observed with a nonexposed cervix. Whether prelabor uterine activity in human beings also affects cervical ripening in the same manner is not completely clear. However, research by Alvarez and Caldeyro[13] documented a gradual increase in prelabor contractility several weeks before birth, the time shown to be associated with cervical ripening in women.[14] It is thus conceivable that prelabor contractility may also be an important factor in cervical ripening in human beings, and that this effect may be in addition to other systemic hormonal influences.

Although PGE_2 has been shown to cause cervical ripening independent of uterine activity,[15] there is ample evidence to confirm that cervical ripening and myometrial maturation for labor occur in concert with each other.[16] Whereas these events may be mostly of an academic interest at term, they could have clinical significance during the preterm period. Namely, if excessive premature low-amplitude uterine contractility can lead to accelerated cervical ripening, inhibition of this type of activity before the onset of active labor could be potentially beneficial. Furthermore, we speculate that if a relationship between prelabor contractility and cervical ripening can be established, investigations to clarify the mechanism behind such a phenomenon could help find treatments to either prevent premature ripening of the cervix or enhance ripening in postdate pregnancies.

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Evaluation of changes in umbilical blood flow in the fetal lamb by Doppler waveform analysis

Klaus G. Schmidt, MD,[a, b] Mariarosaria Di Tommaso, MD,[b] Norman H. Silverman, MD,[a] and Abraham M. Rudolph, MD[a, b]

San Francisco, California

Doppler velocity waveform analysis from descending aortic and umbilical artery signals was performed in 14 instrumented fetal lambs when umbilical blood flow was altered acutely. Blood flow was increased and reduced (by cord occlusion or placental embolization) and ranged 14% to 168% and 8% to 132% from baseline values in the descending aorta and the umbilical artery, respectively. When flow was severely reduced (<50% of baseline value), mean placental resistance increased almost fourfold and mean heart rate fell by 30% from baseline levels. There were only weak correlations between Doppler waveform indices and actual flow, which were usually not significantly different from the correlations between Doppler indices and actual heart rate. With both cord occlusion and placental embolization, significant changes of index values from baseline values were seen only when flow was severely reduced. We conclude that Doppler waveform analysis is not sensitive enough to detect acute mild to moderate reductions in placental flow. Increased index values with severely reduced flow may be explained in part by the concomitant fall in heart rate. (Am J Obstet Gynecol 1991;164:1118-26.)

Key words: Prenatal diagnosis, fetal echocardiography, fetal diseases

Doppler velocity waveform analysis of the umbilical artery[1-3] or descending aortic blood flow[4, 5] has been proposed as a noninvasive method to study the placental circulation in the human fetus. Several indices that are derived from the peak systolic, mean, and end-diastolic velocity are in clinical use, and the method has been widely applied to assess fetal well-being and to predict neonatal outcome.[6-8] Increased indices have been related to reduced umbilical blood flow as a result of increased placental vascular resistance.[9] In clinical studies, the indices that were derived from Doppler waveform analysis have usually been related to markers of fetal well-being or outcome of pregnancy such as fetal growth, pH level, or Apgar score at delivery. However, the mechanisms that may lead to changes of the indices are incompletely understood.

From the Cardiovascular Research Institute, Division of Pediatric Cardiology,[a] and the Department of Obstetrics, Gynecology and Reproductive Sciences,[b] University of California, San Francisco.
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Reprint requests: Abraham M. Rudolph, MD, Room HSE 1403, Box 0544, University of California, San Francisco, San Francisco, CA 94143.
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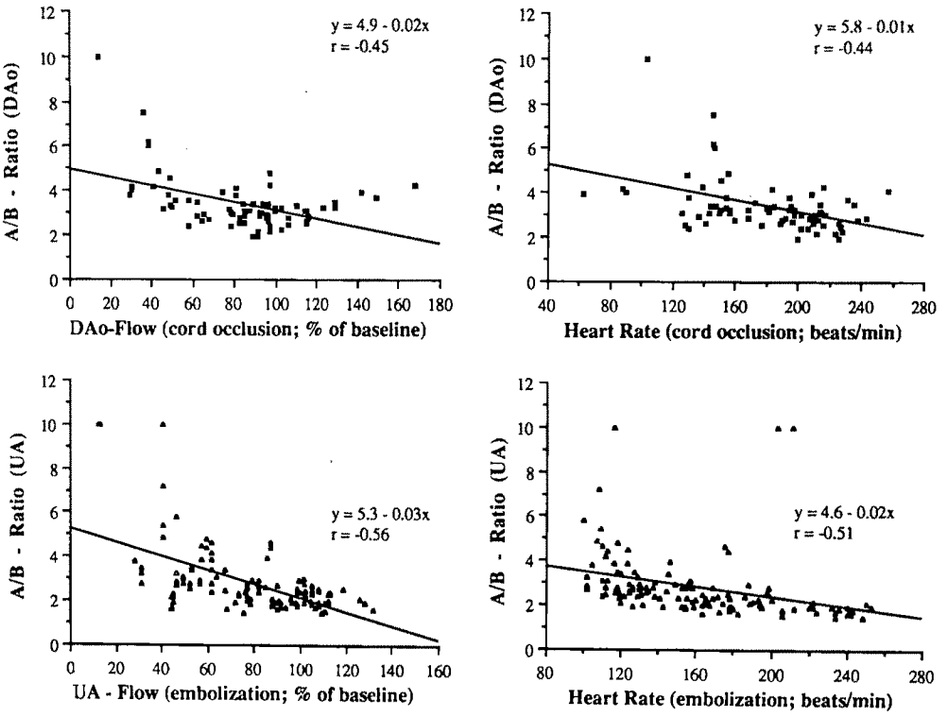


Fig. 1. Examples of the correlation between Doppler waveform indices—here the systolic/diastolic *(A/B)* ratio—and invasively measured blood flow *(left panels)* or fetal heart rate *(right panels)*; *upper panels* refer to descending aortic signals *(DAo)* in the cord occlusion experiment, and *lower panels* refer to umbilical artery signals in the placental embolization experiment. There are weak correlations between index values and reductions of flow, which are similar when index values are related to heart rate.

Only a few experimental studies have approached these questions.[10, 11] We therefore designed this study to investigate the acute influence of alteration of placental flow and resistance on Doppler waveform analysis with three different indices, which were derived from velocity tracings at the descending aortic and umbilical arterial levels.

Methods

Surgical preparation. We studied 14 pregnant sheep of mixed Western breed at 127 ± 3 days of gestation (term = 150 days) with fetuses that weighed 3.0 ± 0.6 kg. The ewes were fasted for 24 hours before surgery. After they had undergone epidural anesthesia by injection of 4 ml of 1% tetracaine, polyvinyl catheters (1.27 mm internal diameter) were placed into a pedal artery and vein of each ewe. Fluids (10% dextrose in 0.9% saline solution, 3 to 5 ml/min) and ketamine (100 mg every 10 to 15 minutes) for maternal sedation were given intravenously. The uterus was exposed through a midline incision, and a hysterotomy was performed on the proximal portion of the pregnant horn. Anesthesia for all fetal surgery was achieved by administration of 0.5% lidocaine. A fetal hind limb was exposed, and polyvinyl catheters (0.76 mm internal diameter) were inserted into the anterior tibial artery and vein and advanced to the lower descending aorta and inferior vena cava, respectively. Next, the other hind limb and rump were extracted through the same hysterotomy, and the left fetal flank was exposed. An incision was made anterior to the spinal muscles along the lower lumbar vertebral column. The abdominal muscles were carefully dissected; the lower descending aorta and common umbilical artery were exposed as described previously.[12]

Two electromagnetic flow transducers (C&C Instruments, Culver City, Calif.) of appropriate size were placed on the descending aorta just proximal to the iliac bifurcation and on the common umbilical artery. In each of eight animals, a polyvinyl catheter (0.4 mm internal diameter) was placed in a small branch of the iliac artery so that its tip was in the common umbilical artery, which allowed an embolization of the placental vascular bed with plastic microspheres; two other cath-

Table I. Linear regression analysis of Doppler waveform indices on fetal descending aortic and umbilical flow or heart rate

Index	*Percent of baseline flow*			*Heart rate*			
	r	*Intercept*	*Slope*	*r*	*Intercept*	*Slope*	*p* Value*
Cord occlusion							
DAo							
A/B	−0.45	4.94	−0.018	−0.44	5.79	−0.013	NS
PI	−0.44	1.75	−0.005	−0.46	2.04	−0.004	NS
RI	−0.38	0.77	−0.001	−0.46	0.85	−0.001	NS
UA							
A/B	−0.36	2.61	−0.019	−0.50	3.44	−0.015	NS
PI	−0.41	0.95	−0.010	−0.54	2.26	−0.007	NS
RI	−0.24	0.60	−0.001	−0.64	0.42	−0.002	NS
Placental embolization							
DAo							
A/B	−0.37	5.10	−0.022	−0.50	6.20	−0.018	NS
PI	−0.42	2.16	−0.009	−0.52	2.56	−0.007	NS
RI	−0.31	0.77	−0.001	−0.64	0.95	−0.002	NS
UA							
A/B	−0.56	5.27	−0.031	−0.51	4.64	−0.018	<0.05
PI	−0.64	1.84	−0.010	−0.68	2.17	−0.007	NS
RI	−0.59	0.81	0.003	−0.61	0.89	−0.002	NS

A/B, Systolic/diastolic ratio; *DAo,* indices obtained from descending aortic velocity waveforms; *PI,* pulsatility index; *RI,* resistance index; *UA,* indices obtained from umbilical artery velocity waveforms.

*Comparison between slopes of regression lines for indices versus flow and indices versus heart rate (*t* test).

eters (0.76 mm internal diameter) were then inserted into a cotyledonary vein and advanced into the central umbilical vein. The other six animals had neither of these catheters inserted, but each had a balloon occluder placed around the umbilical cord. All incisions were closed, and each fetus was returned to the uterine cavity. A catheter with multiple side holes was inserted into the amniotic cavity, and the uterine incision was closed after lost amniotic fluid had been replaced with warmed 0.9% saline solution.

Experimental protocol. The fetuses were studied immediately after surgery. Fluid-filled catheters were connected to Statham P 23Db pressure transducers (Viggo-Spectramed Inc., Critical Care Div., Oxnard, Calif.), which were calibrated to the nearest millimeter of mercury with a static mercury column. Mean and phasic aortic pressures as well as mean amniotic, umbilical, and inferior vena caval pressures were measured. Fetal heart rate was obtained from a cardiotachometer linked to the phasic aortic pressure recording. Mean and phasic descending aortic and umbilical blood flows were monitored with the electromagnetic flow probes with Statham SP 2202 flowmeters. Pressure and flow tracings as well as heart rate were recorded continuously with a Beckman eight-channel direct-writing recorder (Beckman Instruments, Inc., Fullerton, Calif.). A blood gas analysis (model 158, Corning Medical, Medfield, Mass.) was obtained before the study and after each experimental alteration of blood flow.

Measurements were taken at baseline values; then umbilical blood flow was increased by intravenous administration of atropine and norepinephrine (0.1 mg/kg and 1 μg/kg/min, respectively) to the fetus. Subsequently, umbilical blood flow was decreased either by stepwise occlusion of the cord ($n = 6$) or by embolization of the fetal side of the placental vascular bed ($n = 8$) with repeated injections of 2×10^6 plastic microspheres (25 μm in diameter, 3M Company, St. Paul, Minn.) into the common umbilical artery.

Actual descending aortic and umbilical flow was measured at each step with the radionuclide-labeled microsphere technique.[13] Labeled 15 μm microspheres (selected from gadolinium 153, cobalt 57, indium 114, chromium 51, stannum 113, strontium 85, niobium 95, manganese 54, zinc 65; Du Pont Diagnostic Imaging Div., Billerica, Mass.) were injected into the inferior vena cava, while a reference sample was withdrawn from the descending aorta at a rate of 4 ml/min for 1.25 minutes (at least 40 seconds after completion of the microsphere injection). Removed fetal blood was measured and replaced with an equal volume of fetal donor blood or maternal blood.

At the end of the experiment, the ewe and fetus were killed by an overdose of pentobarbital and subsequent bilateral thoracotomy was performed in accordance with the guidelines of the Committee on Animal Research of the University of California, San Francisco. The fetus was weighed and dissected; the lower carcass (below L4-5) and the uterus and cotyledons were weighed and placed in formalin. The tissues were car-

bonized in an oven at 350° C, pulverized, and placed into plastic vials to equal height. The radioactivity of tissues and reference blood samples was counted in a 1000-channel multichannel pulse-height analyzer (Norland Corp., Fort Atkinson, Wis.). The specific activity of each isotope within a sample was calculated by the least-squares method.[14] Descending aortic and umbilical blood flow (Q) was calculated according to the formula: Q = (Reference flow × Organ counts)/Reference counts. Electromagnetic flowmeter readings of descending aortic and umbilical flow were adjusted to the microsphere measurements; they served as flow measurement at any time between microsphere determinations. In lambs that underwent placental embolization, the peripheral vascular resistance (R) was calculated as: R = (Mean aortic − Mean inferior vena caval pressure)/Flow.

Doppler ultrasonographic study. Pulsed Doppler interrogation of the descending aorta and the umbilical artery was performed several times at each step of altered descending aortic and umbilical flow. We used an ATL Mark 600 instrument (Advanced Technology Laboratories, Inc., Bothell, Wash.) with a 5 or 3 MHz mechanical transducer that was placed directly on the uterine horn that contained the instrumented fetus. Images of the fetal descending aorta and the umbilical cord were obtained in all animals. The Doppler sample volume was placed in the lower descending aorta or in the umbilical cord close to the site at which it enters the fetal body until high-pitched acoustic signals of the pulsatile flow were heard, and an optimal signal was displayed by the spectral output. Ultrasonographic evaluation was recorded continuously on half-inch videotape for later analysis. Time registration by means of recording paper speed and the internal clock of the ultrasonographic machine allowed off-line comparison of the Doppler indices with the measured physiologic variables at one time.

Off-line analysis of the Doppler tracings was performed with a computer-assisted analysis system and commercially available software (CAD 886, Microsonics, Indianapolis). Peak systolic, least diastolic, and mean velocities were measured from the maximum spectral display of the Doppler shift that was sampled in the fetal descending aorta and umbilical artery. Doppler waveform indices were calculated as:

Systolic/diastolic (A/B) ratio = Peak systolic/End-diastolic velocity

Pulsatility index = (Peak systolic − End-diastolic velocity)/Mean velocity

Resistance index = (Peak systolic − End-diastolic velocity)/Peak systolic velocity

Five consecutive beats were averaged for each index determination. For the systolic/diastolic ratio, a value of 10 was considered maximum and was also assumed if end-diastolic velocity was zero.

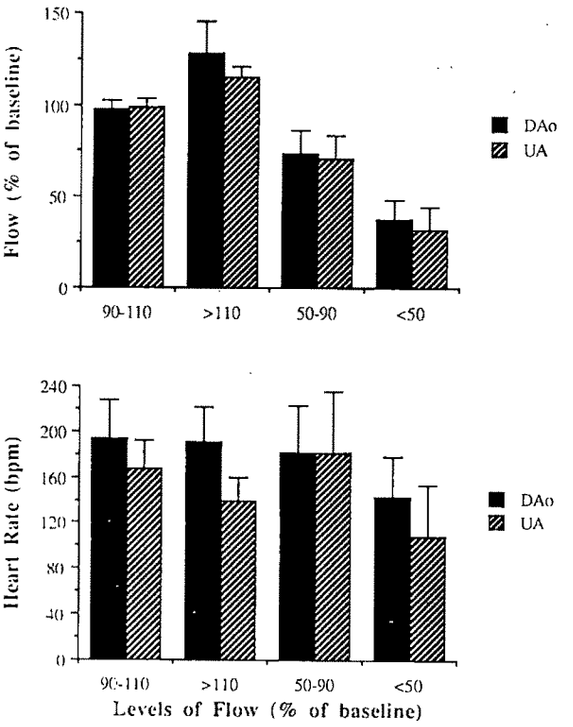


Fig. 2. In the cord occlusion experiment, increases and reductions of mean flow (+SD) at the three levels of altered flow that were measured in both the descending aorta *(DAo)* and umbilical artery *(UA)* are shown *(top)*. Simultaneous changes in heart rate also are shown *(bottom)*.

Statistics. Data are given as mean ± SD. The descending aortic and umbilical blood flow alterations were expressed as percent from baseline flow in each animal. Linear regression analysis was used to correlate both blood flow and heart rate with Doppler waveform indices that were obtained simultaneously. Student's *t* test was used to compare the slopes of the regression lines.

For further analysis the actual flow measurements were assigned to one of four levels of flow: 90% to 110% was considered to be baseline flow, >110% increased flow, 50% to 90% moderately decreased flow, and <50% severely decreased flow. Measured physiologic variables and calculated Doppler waveform indices were pooled according to these levels of flow both in the cord occlusion and in the placental embolization protocols. Analysis of variance was used to assess differences at the different levels of flow for each Doppler waveform index. If the null hypothesis had to be rejected, multiple comparisons between different levels

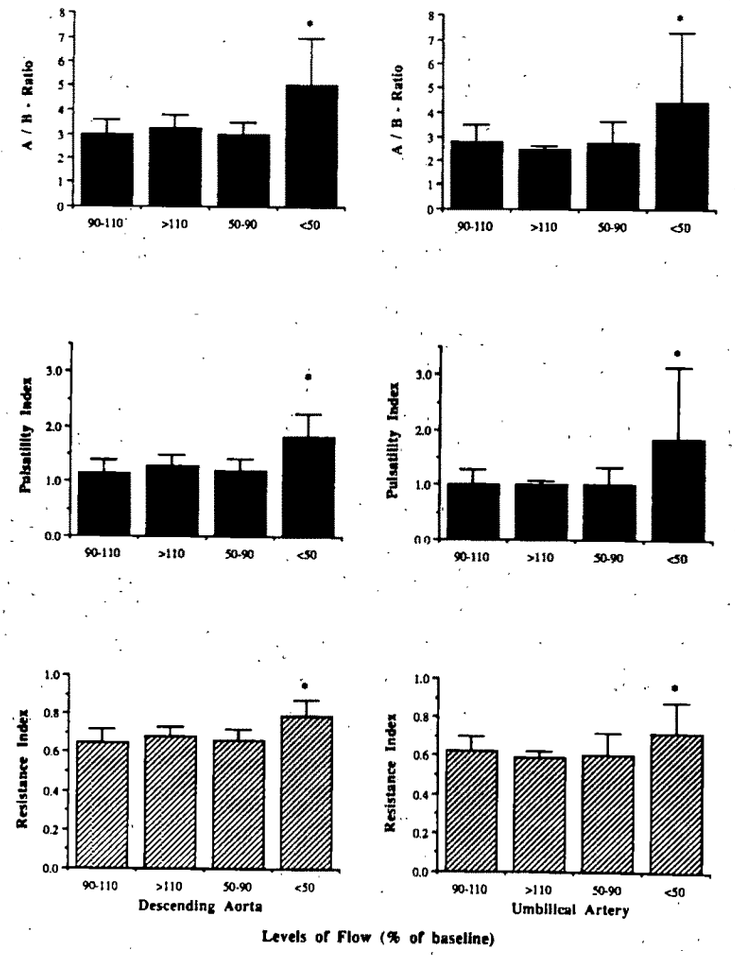


Fig. 3. Doppler waveform indices in the cord occlusion experiment as assessed at baseline and at three levels of altered flow in both the descending aorta *(left)* and umbilical artery *(right)*. Indices shown are systolic/diastolic *(A/B)* ratio *(top)*, pulsatility index *(middle)*, and resistance index *(bottom)*. Significant changes from baseline only occurred in the group with severely reduced flow *(asterisk,* $p < 0.05$).

of flow were made by the Scheffé method; $p < 0.05$ was considered to indicate a significant difference between groups.

Results

Cord occlusion. In the cord occlusion experiment, blood flow alterations, which were expressed as percent from baseline flow, ranged from 14% to 168% in the descending aorta and from 8% to 124% in the umbilical artery. The three Doppler waveform indices that were tested showed considerable variability at all levels of flow; with severely reduced flow, index values tended to increase. There were weak correlations between flow and index values over the range of flow alteration (Fig. 1, top left). There was, however, a similar trend if index values were correlated with heart rate (Fig. 1, top right). The slopes of the regression lines for index values versus blood flow did not differ significantly from those for index values versus heart rate (Table I).

At each level of alteration, mean flow was significantly different from baseline flow; with severely compromised flow, it was decreased to 38% ± 10% from baseline in the descending aorta and to 34% ± 13% from baseline in the umbilical artery (Fig. 2). Concomitantly, there was a significant fall in heart rate from 194 ± 34 to 143 ± 35 beats/min (descending aorta) and from 181 ± 55 to 108 ± 45 beats/min (umbilical artery) when the baseline and severely reduced flow levels were compared. Over these ranges of altered blood flow, Doppler waveform indices were determined on 74 oc-

casions from descending aortic velocity tracings and on 84 occasions from umbilical arterial velocity tracings. Significant changes of the three Doppler waveform indices from their respective baseline values were only noted when flow was reduced to <50% of baseline (Fig. 3); this observation was made for the Doppler indices that were obtained from both the descending aortic and umbilical artery velocity tracings.

Placental embolization. In the placental embolization experiment, blood flow alterations ranged from 21% to 135% in the descending aorta and from 13% to 132% in the umbilical artery. As in the cord occlusion experiment, there were weak correlations between flow and index values over the range of flow alteration, and index values also correlated weakly with heart rate (Fig. 1, bottom). The slopes of regression lines that correlated index values with blood flow or heart rate did not differ significantly except for the systolic/diastolic ratio, which was obtained from umbilical artery signals (Table I). At each level of alteration, mean flow was significantly different from baseline flow. At the severely compromised flow level, it decreased to 39% ± 10% from baseline in the descending aorta and to 42% ± 9% from baseline in the umbilical artery (Fig. 4). Concomitantly, there was a significant, almost fourfold increase in the peripheral vascular resistance and significant decreases in fetal heart rate and fetal pH (Fig. 4). Mean heart rates fell from 169 ± 36 beats/min at baseline to 131 ± 27 beats/min (descending aorta) and from 178 ± 30 to 131 ± 29 beats/min (umbilical artery) when flow was severely reduced (<50% of baseline). Over the ranges of altered blood flow, Doppler waveform indices were determined on 72 occasions from descending aortic velocity tracings and on 129 occasions from umbilical arterial velocity tracings. Significant mean changes of the three Doppler waveform indices from their respective baseline values were only noted when flow was reduced to <50% of baseline (Fig. 5); this observation was made for the Doppler indices that were obtained from both descending aortic velocity tracings and umbilical artery velocity tracings.

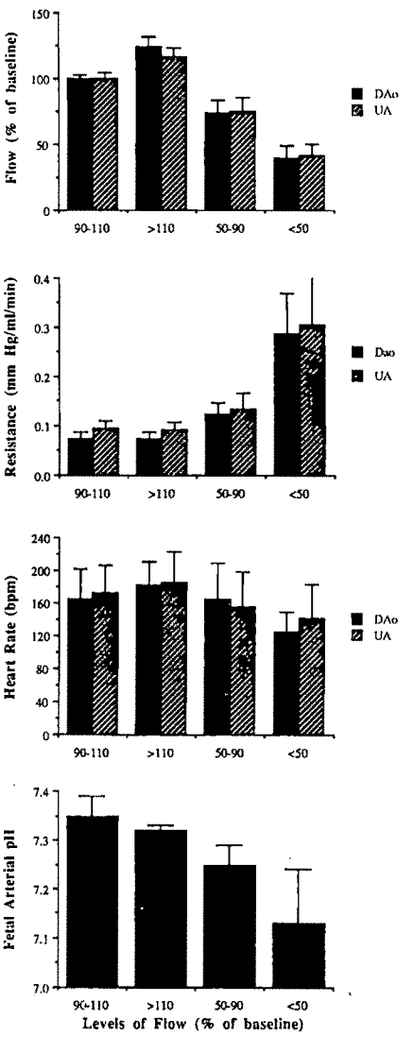


Fig. 4. In the placental embolization experiment, increases and reductions of mean flow (+SD) at three levels of altered flow that were measured in both the descending aorta *(DAo)* and umbilical artery *(UA)* are shown *(top)*. Simultaneous increase in placental vascular resistance *(upper middle)* and decrease in heart rate *(lower middle)* are also shown. With reduction in blood flow, fetal arterial pH values were significantly reduced *(bottom)*.

Comment

Our study demonstrates that Doppler waveform indices that are obtained from descending aortic and umbilical arterial velocity tracings are not sensitive enough to detect mild to moderate acute changes of blood flow. Although we altered umbilical blood flow considerably (Figs. 2 and 4), mean index values that were obtained both from descending aortic signals and umbilical arterial signals changed significantly from baseline values only when blood flow was severely reduced to <50% of baseline; a similar observation was made when umbilical blood flow was reduced both by venous and by arterial obstruction of the fetal side of the placental circulation when mechanical occlusion to the cord or arterial embolization was employed (Figs. 3 and 5).

Both modes of obstruction to umbilical blood flow may occur and may lead to placental dysfunction in human fetuses as well. Whereas cord occlusion causes venous congestion of the placental vascular bed, embolization causes obstruction at the level of small co-

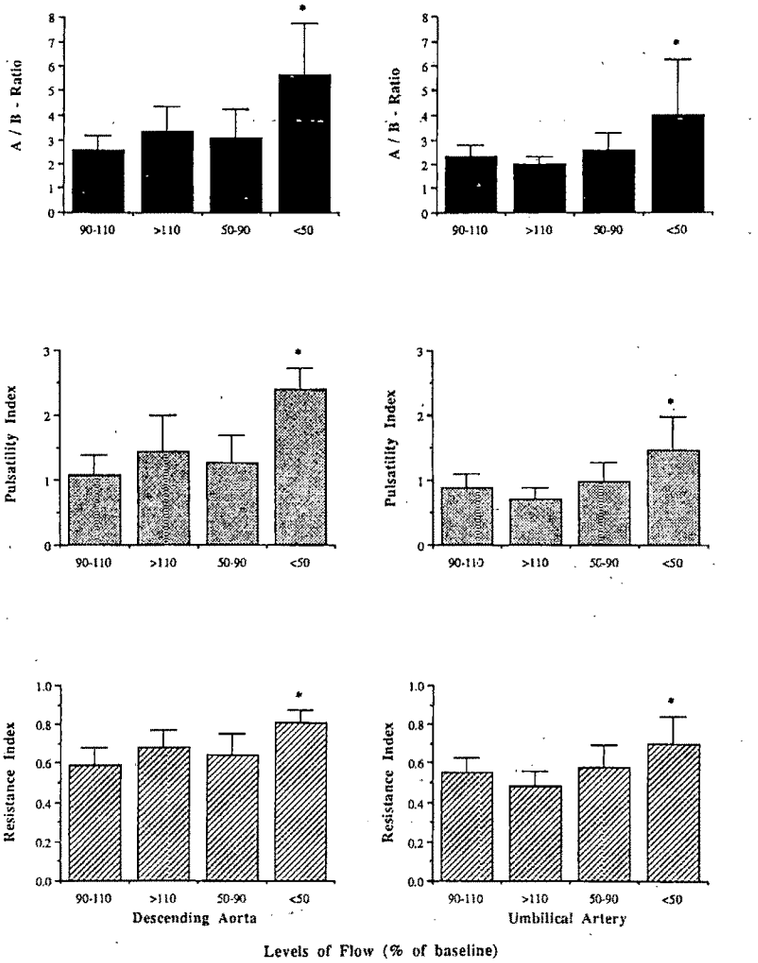


Fig. 5. Doppler waveform indices in the placental embolization experiment as assessed at baseline and at three levels of altered flow in both the descending aorta *(left)* and umbilical artery *(right)*. Indices shown are systolic/diastolic *(A/B)* ratio *(top)*, pulsatility index *(middle)*, and resistance index *(bottom)*. Significant changes from baseline occurred only in the group with severely reduced flow (*asterisk*, $p < 0.05$).

tyledonary arteries with a concomitant increase in placental vascular resistance. In human fetuses the suggestion has been frequently made that increased placental vascular resistance causes increased Doppler waveform indices.[1-6] High placental resistance was attributed to a loss of small arteries that are found in the placenta when high index values had been observed in these fetuses.[9] In our embolization experiment, however, moderate increases in placental vascular resistance, which raised it to a mean of 140% to 165% from baseline value, did not significantly change the Doppler waveform indices. This finding suggests that early changes in the placental vascular bed that lead to an increase in resistance are not detected by Doppler waveform analysis.

Only after an increase in resistance to almost 400% of baseline level did mean index values increase significantly, but even then, many index values were still found in the range that is seen at the baseline level (Fig. 1). Accordingly, the standard deviations of index values in the severely compromised flow groups increased considerably (Figs. 3 and 5). Sometimes we also observed high index values at the baseline flow level; other reports have related such variability of Doppler waveform indices to the use of different instruments for the ultrasonographic studies[15] or to considerable interobserver variability,[16] but we used the same ultrasonographic machine for all studies and had one observer, who was blinded to actual flow measurements, interpret all of the studies. Whatever the cause, this study dem-

onstrates a considerable variability of Doppler waveform indices at normal and compromised flow levels, which limits its usefulness in detection of the fetus that is at impending risk for the development of significant placental dysfunction.

In the recent experimental study by Trudinger et al.,[10] an observed increase in the systolic/diastolic ratio was related to a moderate rise of the placental vascular resistance as a result of chronic embolization of the fetal umbilical arteries. In this study, however, the mean values of the systolic/diastolic ratio ranged between 2 and 2.5 both in control and treated animals; these values represent a normal range that would not allow identification of a condition of increased placental vascular resistance in a general population. The authors also state that heart rates in control and treated animals did not change over the course of the experiment.[10] This finding is very different from our own and those of other researchers,[11] who observed a decreasing fetal heart rate after placental embolization; this discrepancy remains unexplained. According to our results, heart rate is significantly related to an increase of index values when umbilical flow is reduced. The weak correlations that we found between actual flow and simultaneous index values were similar to those that were obtained when index values were plotted against heart rate; most commonly, the slopes for these regression lines were not significantly different from each other, which suggests that increased index values can also be related to the reduced heart rate.

Several clinical reports have linked increases in Doppler waveform index values mainly to simultaneous slowing in heart rate.[17, 18] This seems reasonable because the fetus with a slower heart rate has a longer diastolic period and a lower end-diastolic velocity in the descending aorta or umbilical artery; lower end-diastolic velocity, in turn, will lead to higher index values. Other researchers, however, have found little[19] or no effect[20] of fetal heart rate on Doppler waveform indices. Van Huisseling et al.[21] recently demonstrated in fetal lambs that pulsatility index increased during fetal hypoxemia that was induced by obstruction of the maternal common iliac artery without change in placental resistance; however, pulsatility index remained unchanged when fetal heart rate was held constant by parasympathetic blockade. The authors conclude that increasing index values are mainly related to slowing of fetal heart rate but not to placental resistance.[21]

Among all combinations tested, the index values that were obtained from umbilical artery velocity signals in the embolization experiment showed the best correlation with actual flow (Table I). This may be significant for the clinical situation in which umbilical arterial signals rather than descending aortic signals should be used; umbilical arterial Doppler signals may also be found more easily than descending aortic signals. Furthermore, all three indices that were tested provided similar results when different modes of flow reduction and variable sites of Doppler interrogation were considered. Therefore there seems to be no special advantage in the use of one index instead of another. Because the systolic/diastolic ratio requires minimal computation, it may be the best index to use in the clinical situation, if there is indeed a need to do so. The parameters that determine vascular resistance are complex and multifactorial. Because the calculation of fetal descending aortic and umbilical blood flow with the Doppler technique has been shown to be reasonably accurate[22] and useful in the clinical situation,[23] such flow measurements may provide an alternative to the Doppler waveform indices.

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Immunohistochemical localization of gonadotropin-releasing hormone during implantation in the New Zealand White rabbit

Paul A. Bergh, MD,[a] Ted L. Anderson, PhD,[b] and Glen E. Hofmann, MD, PhD[a]

New York, New York, and Nashville, Tennessee

Gonadotropin-releasing hormone was localized immunohistochemically during implantation (gestational days 6 to 14) in the New Zealand White rabbit. During early implantation (days 7 to 9), intense gonadotropin-releasing hormone immunostaining was localized predominantly to the cytoplasm of the nonknob cytotrophoblast with light to moderate staining in the cytoplasm of the syncytiotrophoblast (knob). In later gestation, light to moderate staining of the cytoplasm of the trophoblast at the true placental site was detected. No appreciable change in staining was noted after day 10. Fetal membranes, identified after day 10, showed intense and unchanging immunostaining for gonadotropin-releasing hormone. Obplacental giant cells showed light to moderate nuclear and cytoplasmic gonadotropin-releasing hormone immunostaining. Light to moderate gonadotropin-releasing hormone immunostaining was also noted in the cytoplasm of uterine epithelium and glands. We conclude that immunoreactive gonadotropin-releasing hormone is present in the cytotrophoblast at the time of the earliest embryo-uterine interactions and may play a significant role in implantation and embryo survival. (AM J OBSTET GYNECOL 1991;164:1127-31.)

Key words: Gonadotropin-releasing hormone, implantation, trophoblast, immunohistochemistry

The decapeptide, gonadotropin-releasing hormone (GnRH), is found in the arcuate nucleus of the hypothalamus, and its pulsatile release controls gonadotropin secretion from the pituitary gland.[1] A GnRH-like substance that is immunologically and biologically similar to hypothalamic GnRH has been shown in the placenta of many species including rabbits and human beings.[2,3] In human placenta, this GnRH-like substance appears to be involved in the control of the synthesis and secretion of the gonadotropin, human chorionic gonadotropin (hCG).[4-6]

A paracrine function has also been postulated for GnRH in the rabbit related to early embryo-uterine interaction and nidation[7]; hence it would be useful to study the presence of GnRH-like substance in the periimplantation and early postimplantation trophoblast as the embryo prepares to adhere to and implant in the uterine epithelium. For obvious practical and ethical reasons, studies involving the use of periimplantation trophoblast cannot be carried out in human subjects. Therefore because the temporal, physiologic, and morphologic aspects of nidation are well characterized in the rabbit,[8-10] this model was used to localize and describe the expression of GnRH-like activity during implantation. A distinctive feature of the rabbit blastocyst is the appearance of discrete islands of trophoblastic knobs (syncytiotrophoblast) dispersed throughout the cytotrophoblast (nonknob). Previous studies localized GnRH-like activity in human placenta predominantly to the cytotrophoblast, along the outer surface layer of the syncytiotrophoblast, and in the villous stroma.[11] Thus the configuration of the rabbit placenta provides a unique opportunity to examine potential roles for GnRH in the initial stages of trophoblastic differentiation and implantation. Immunohistochemistry was used to characterize the expression of a GnRH-like substance by the very earliest trophoblast and to clarify a possible role in embryo-uterine interactions.

From the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Mount Sinai Medical Center,[a] and The Vanderbilt Medical School.[b]

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Reprint requests: Glen E. Hofmann, MD, PhD, Department of Obstetrics and Gynecology, Box 1175, Mount Sinai Medical Center, 1 Gustave L. Levy Place, New York, NY 10029.

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Material and methods

Adult New Zealand White rabbits were used for these studies. They were caged individually in an American Association of Accreditation of Laboratory Animal Care–accredited facility on an alternating 12-hour light and dark photography period with free access to food and water. Mating of the animals, uterine perfusion and fixation, and implantation site processing for light microscopy and immunohistochemistry were performed as previously described.[12] The day of mating was defined as day zero.

Immunohistochemical localization of GnRH-like fac-

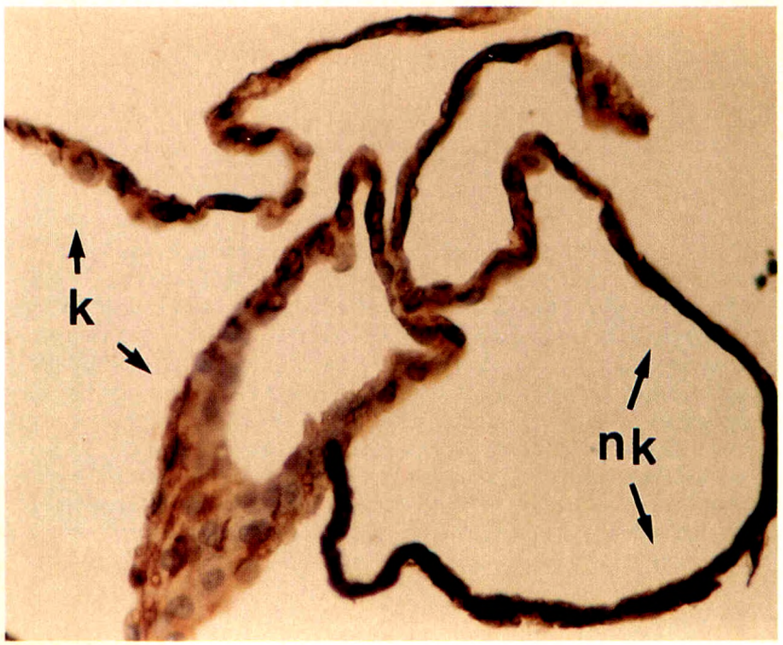


Fig. 1. Early implantation site (day 8), GnRH immunostaining is very intense and is localized predominantly to the cytoplasm of cytotrophoblast (*nk*). Moderate staining of the cytoplasm of the syncytiotrophoblast (*k*) is also noted. (×350).

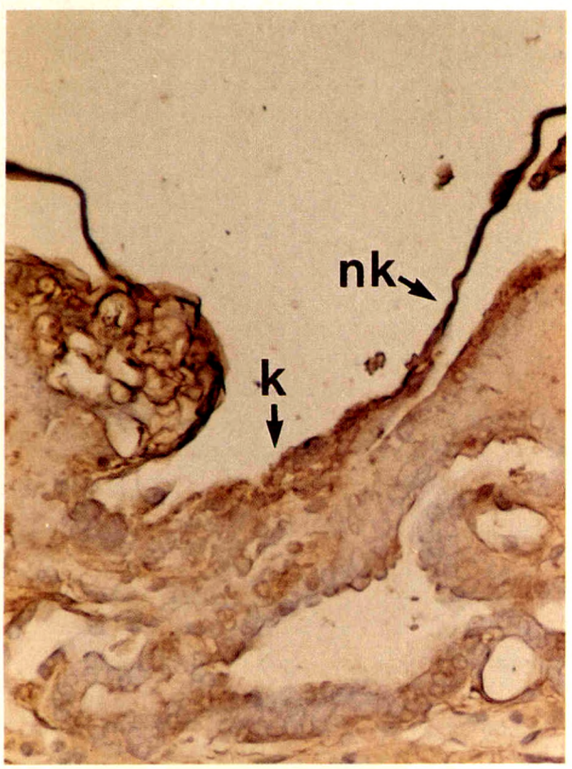


Fig. 2. Early implantation site (day 8). Differential staining of cytotrophoblast (*nk*) is maintained as the syncytial knob (*k*) fuses with uterine epithelium. Knob shows only light staining. Note staining of the uterine epithelium and glands. (×350).

tor was accomplished on 5 μm paraffin-embedded sections with polyclonal sheep anti-GnRH No. 94 (generously provided by Dr. H. M. Fraser, Edinburgh, Scotland). The specificity of this antibody is directed against the C-terminal end of GnRH. Cross-reactivity with fragments of GnRH remained at 100% after the removal of up to 3 N-terminal amino acids, whereas cross-reactivity dropped to <0.01% with the replacement at position 10 of Gly-NH_2 by Gly-OH or with the use of other brain peptides.[13] The specificity of this antibody against the high molecular form(s) of GnRH has not been established (unpublished observation). Immunohistochemical localization of the GnRH-like substance was performed by the ABC method (Vector Laboratories, Burlingame, Calif.) with the peroxidase substrate, 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis). The protocol used was as follows: endogenous peroxidases were blocked with 1% hydrogen peroxide in methanol. Nonspecific binding was blocked by incubation with 10% normal serum. Tissues were then incubated overnight (4° C) with the primary antibody diluted to 1:1000 in phosphate-buffered saline solution with 5% bovine serum albumin (Sigma Chemical Co.). The tissues were then washed in phosphate-buffered saline solution and the biotinylated antibody applied. The slides were then lightly counter-stained with hematoxylin. In control incubations, identical sections were treated in the same fashion except that the primary (anti-GnRH) or secondary (biotinylated) anti-

body were excluded or the primary antibody was preabsorbed with the native GnRH (Sigma Chemical Co.) at a final concentration of 1 μmol/L. First-trimester human placenta served as a positive control. In addition, antiserum was diluted in phosphate-buffered saline solution with 1%, 5%, 10%, and 15% bovine serum albumin to assess the possibility that the antiserum recognized the bovine serum albumin to which the GnRH was conjugated during immunization of the sheep. At least two animals were studied at each gestational day (6 through 14) and at least two different implantation sites from each animal were examined.

Immunohistochemical staining was recorded as absent, light, moderate, or intense. All results were correlated with the gestational age of the implantation sites.

Results

Immunoreactivity for the GnRH antibody was detected in all sections from each gestational day studied. In early implantation sites (days 7 to 9), GnRH immunostaining was very intense and was localized predominantly to the cytoplasm of the nonknob cytotrophoblast. Light to moderate staining was noted in the cytoplasm of the syncytiotrophoblast (knob); however, no nuclear staining was detected (Fig. 1). This differential staining pattern was maintained after fusion of the syncytial knob with the uterine epithelium (Fig. 2). In later implantation sites (days 10 to 14), light to moderate GnRH immunostaining was identified in the true placenta (Fig. 3). Fetal membranes (identified from day 10 onward) stained more intensely than did the placenta (Fig. 3). Immunostaining of placenta and fetal membranes was unchanged on all gestational days studied (days 10 to 14). Obplacental giant cells showed light to moderate nuclear and cytoplasmic immunoreactive GnRH staining (Fig. 4). Light to moderate GnRH immunoreactivity was detected in the uterine surface epithelium, stroma, and glands (Fig. 2) both before and after fusion of the trophoblast with uterine epithelium. Immunostaining was not observed in nonimplantation sites, resorption sites, and nonpregnant uteri. Also, no immunostaining was seen in the control experiments in which either antibody was excluded or the primary antibody was preabsorbed with GnRH. First-trimester human placenta showed intense GnRH immunostaining as described previously.[11] Immunostaining was not affected by changes in the percentage of bovine serum albumin, suggesting the antibody did not recognize any portion of the bovine serum albumin (not shown).

Comment

In human beings, exogenous GnRH has been found to stimulate production of hCG and release by placental tissue and cells in vitro.[4-6] The hCG produced by the placenta is required to rescue the corpus luteum thus ensuring adequate progesterone production needed for complete endometrial decidualization and successful nidation. Immunofluorescence has located the placental GnRH-like peptide predominantly to the cytotrophoblast, making this the most likely site of production.[11] Thus it is theorized that placental GnRH or a related substance derived from the cytotrophoblast may effect hCG production and secretion by acting on trophoblast receptors perhaps located on the syncytiotrophoblast cell membrane.[11] Further support of this relationship is offered by the observation that maternal serum levels of hCG correlate very closely with the existing number of cytotrophoblasts. The localization of GnRH along the outer surface of syncytiotrophoblast cell membrane is consistent with the mechanism of action of a peptide hormone.[14] These findings led to speculation that the placenta may function in a manner analogous to the hypothalamic-pituitary-gonadal axis and may possess its own GnRH-mediated hCG regu-

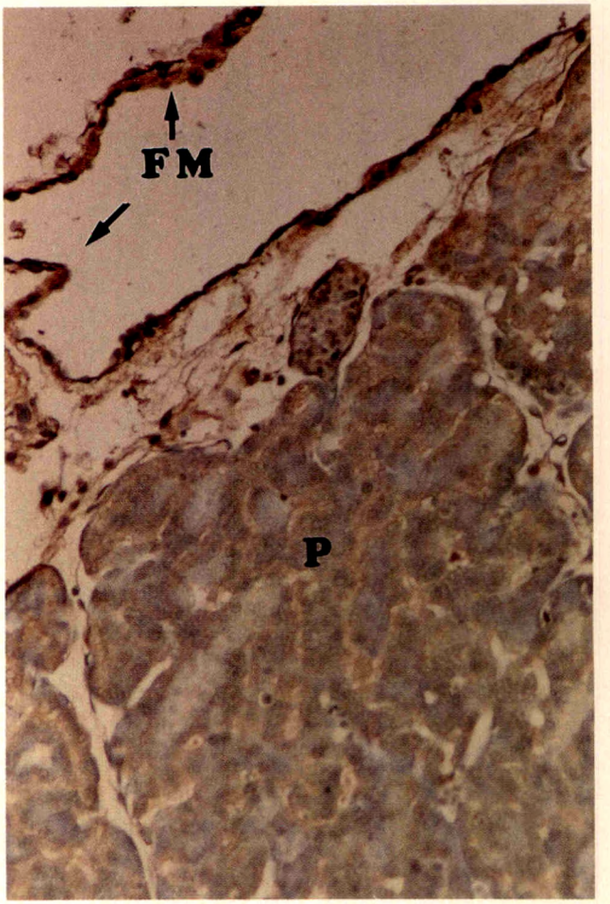


Fig. 3. Late implantation site (day 13) shows light to moderate GnRH immunostaining of true placenta (*P*). Fetal membranes (*FM*) stain more intensely than placenta. (× 145).

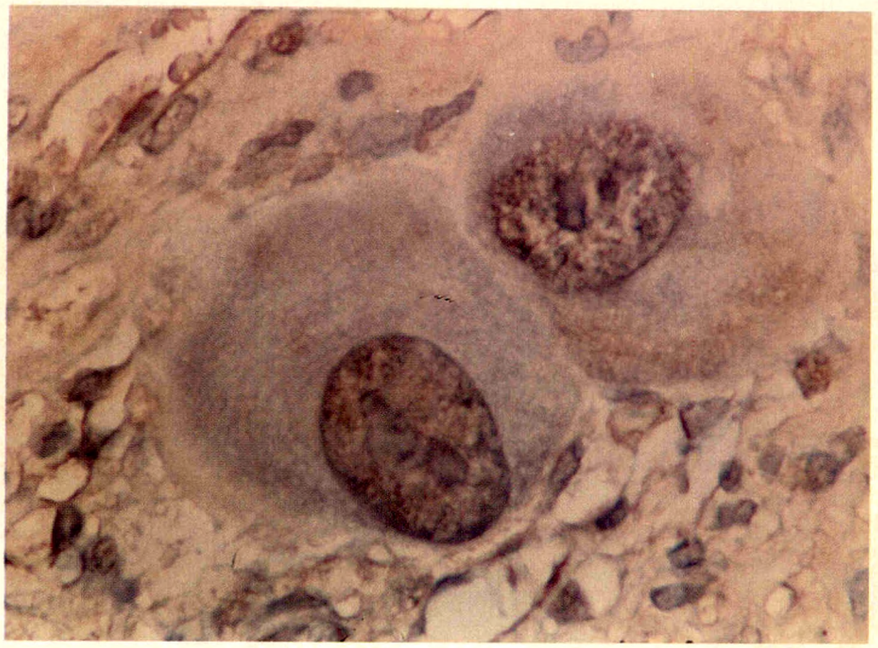


Fig. 4. Obplacental giant cells show light to moderate nuclear and cytoplasmic immunoreactive GnRH staining. (×710).

latory system. In addition, GnRH may play a role in a paracrine system related to local embryo-uterine interactions and nidation.

The physiologic function of this GnRH-like factor in the rabbit remains to be determined. Unlike in human beings, the mechanism by which the embryo maintains corpus luteum function is not clear. Browning et al.[15] failed to show any chorionic gonadotropin activity in rabbit placenta obtained on days 10, 18, and 26 of gestation. Gonadotropin-like activity was found to be greater in peripheral serum versus serum from the uterine vein. In addition, after removal of the conceptus at day 18, the corpus luteum of pregnant rabbits could be maintained with injections of steroid-free pituitary extracts or estrogen.[16] Although Nowak and Bahr[7] showed in vitro placental secretion of a GnRH-like factor in the rabbit, they found no difference in concentration between the uterine venous blood or peripheral blood. These studies therefore suggest that the GnRH-like substance secreted by the rabbit placenta acts locally and may be involved in early embryo-uterine signaling.[6, 7]

The immunostaining pattern of GnRH in the rabbit differs from that of the human being as well. Both the rabbit and human being[11] show intense GnRH immunoreactivity in cytotrophoblast. However, the staining pattern of the syncytiotrophoblast differs from that in human beings because immunoreactive GnRH is found only on the surface of the syncytiotrophoblast, whereas in rabbits there is no detectable GnRH immunoreactivity on the surface of the syncytiotrophoblast. In contrast, the GnRH immunostaining in the rabbit is found in the cytoplasm of the syncytial knobs. This difference could be because of the difference in species, the difference in the model studied (rabbit, in vivo perfusion; human being, curettage specimen) or to the difference in the physiologic role(s) for GnRH in human beings and rabbits. The length of gestation in the rabbit is 30 days and the distinction between cytotrophoblast and syncytiotrophoblast is lost after day 9 when the true placenta is formed. Whereas our study may cover a relatively wide gestational period, both our study and previous human studies cover the early part of gestation and should be comparable as far as interspecies differences allow. Finally, GnRH immunostaining was noted both before and after fusion of the trophoblast with the uterine epithelium. This suggests that the rabbit epithelium may contain a GnRH-like substance as in human beings.[17]

The GnRH immunostaining in obplacental giant cells is of interest. The exact origin of these cells is debated, but a recent study suggests that they are derived from the syncytiotrophoblast.[18] In addition, some researchers believe that the obplacental cells are luteotropic in the rabbit.[19] Immunoreactive GnRH was detected in the nucleus and cytoplasm of obplacental giant cells. The finding of immunoreactive GnRH on giant cells is consistent with the hypothesis[19] that they originate from

the syncytiotrophoblast and their luteotropic role in the rabbit. The significance of the nuclear staining is unknown. Further work in this area is needed.

In this study we show the presence of GnRH or a GnRH-like substance in the periimplantation and early postimplantation rabbit cytotrophoblast as the embryo prepares to adhere to and implant in the uterine epithelium. This finding lends further support for a proposed paracrine function for GnRH in both the human being and rabbit related to early embryo-uterine interaction and nidation.

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Passive membrane properties and inward calcium current of human uterine smooth muscle cells

Roger C. Young, MD, PhD, Lea Herndon Smith, and Nels C. Anderson, Jr., PhD
Durham, North Carolina, and Charleston, South Carolina

Human myometrium was obtained at the time of cesarean delivery by means of excisional biopsy from the upper margin of the uterine incision through the lower uterine segment. Isolated smooth muscle cells were obtained by collagenase treatment. With the whole-cell patch clamp technique, voltage-clamp and current-clamp experiments were performed. Regenerative action potentials were seen in 3 mmol/L calcium bathing solution. Voltage-clamp experiments demonstrated the presence of transient inward currents and large outward currents. Inward currents were isolated with the use of tetraethylammonium ion as a blocking agent of outward current. In 3 mmol/L calcium inward current began at −60 mV and maximum inward current occurred at −40 mV. Maximum inward current density during the rising phase of the action potential was 1.0 μA/cm² in 3 mmol/L calcium and was unaffected by reduction of sodium concentration in the bathing solution. (Am J Obstet Gynecol 1991;164:1132-9.)

Key words: Human uterine smooth muscle, electrophysiology, voltage-activated calcium channel

Membrane excitability properties play an important role in the control of uterine contractility.[1] The rise in intracellular calcium that is necessary for smooth muscle contraction is a direct result of an action potential, which activates calcium ion flow across the surface membrane and possibly triggers release of intracellular calcium stores.[2-4]

Single-channel calcium conductances have recently been observed[5] in human uterine tissue with the use of the patch clamp technique.[6] The observation of two distinct conductance levels implied the existence of two types of calcium channels. We report here a characterization, including activation and inactivation properties, of the whole-cell calcium currents that were observed in human uterine smooth muscle cells freshly isolated from term pregnant women.

Material and methods

Preparation of cells. Cell isolation techniques were similar to those reported previously.[7] Small strips of human myometrium (0.5 cm × 0.5 cm × 2.0 cm) were excised from the upper margin of the uterine incision through the lower uterine segment at the time of cesarean delivery (Medical University of South Carolina Institutional Review Board Approval No. 3605). The tissue was transported and stored for up to 2 hours in 120 mmol/L sodium chloride, 5 mmol/L potassium chloride, 10 mmol/L HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethansulfonic acid) buffer at pH 7.2. The serosa and endometrium were dissected free and discarded. The myometrium was minced into 1 mm cubes and washed in calcium-free, magnesium-free Hank's balanced salt solution (Sigma Chemical Co., St. Louis). The washed myometrial pieces were placed into an enzyme solution that was composed of 0.4% crude collagenase (type CLS 3, Worthington, Freehold, N.J.) in calcium-free magnesium-free Hank's balanced salt solution and agitated at 35° C for 20 minutes. The tissue was then placed into fresh enzyme solution under the same conditions for 30 minutes. The enzyme solution was then removed and the partially digested tissue was gently washed in 120 mmol/L sodium chloride, 5 mmol/L potassium chloride, 10 mmol/L HEPES buffer at pH 7.2. Free cells were liberated by repeated trituration of the tissue through a Pasteur pipette. These freshly dispersed cells are large, typically 150 to 300 μm in length and 10 to 15 μm in width. An aliquot of solution that contained free cells was then placed into the recording chamber. Most cells appeared to be elongated, with length-to-width ratios of approximately 20:1, and exhibited tapered ends. Cells with rounded ends or low length-to-width ratios were not considered appropriate for study.

Recording. The myometrial cells were allowed to settle and adhere to the glass bottom of the recording chamber. The recording chamber was then flooded

From the Division of Physiology, Department of Cell Biology, Duke University Medical Center, and the Department of Obstetrics and Gynecology, Medical University of South Carolina.
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Reprint requests: Roger C. Young, MD, PhD, Department of Obstetrics and Gynecology, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425.
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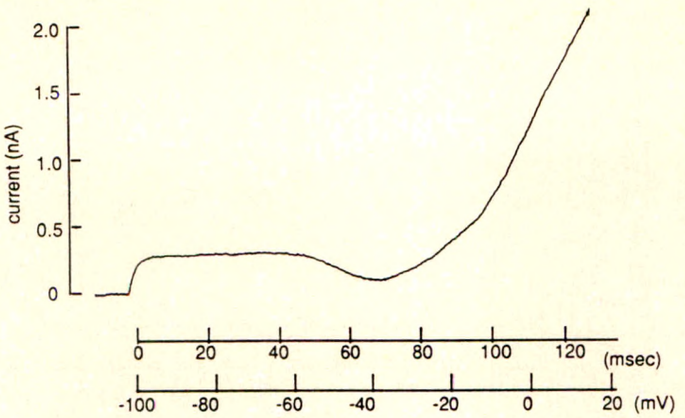


Fig. 1. Whole-cell voltage-clamp experiment. Current responses to voltage ramp. Bathing solution 1 (3 mmol/L calcium, 0 mmol/L TEA^+). Holding potential is −100 mV.

with an electrolyte bathing solution that was selected from the group of solutions in Table I. Cells were observed with a Zeiss Axiovert 35 inverted phase-contrast microscope (Carl Zeiss Inc., Thornwood, N.Y.).

Electrodes were pulled on a horizontal puller (Flaming-Brown, Sutter Instruments, San Rafael, Calif.) with borosilicate capillary tubes. Electrode resistances ranged from 4 to 7 MΩ when filled with electrolyte solution (solution 3, Table I).

Patch clamp recordings were performed with the Axopatch 1-B amplifier (Axon Instruments, Foster City, Calif.). A 1 GΩ feedback resistor was used, and data were filtered through a four-pole Bessel filter that was set at 2 kHz cutoff. After formation of a gigaohm seal between the patch pipette and the cell membrane, intracellular access was accomplished with moderate suction on the pipette at a holding potential of −60 mV under voltage-clamp conditions.

Data were digitized with the Axolab 1100 data acquisition system (Axon Instruments) and analyzed with the p-CLAMP software (Axon Instruments) on a Compaq 286 computer (Compac Computer Corp., Houston). All recordings were made at 20° to 25° C. Electrode capacitance and stray capacitance were electrically compensated; however, capacitive currents as a result of cell membrane capacitance were not compensated.

Voltage-clamp data were obtained by application of voltage-step or voltage-ramp commands from the holding potential to test potentials while the membrane current was monitored. By standard convention, positive ions that flow into the cell are defined as inward current and are represented by a downward deflection of current. The magnitude and time course of membrane currents were stable over time (up to 1 hour). An interval of at least 2 seconds between test pulses was necessary for the cell to reequilibrate and to avoid inactivation of membrane currents. For data reported here, 5 seconds was generally allowed as a convenient interval between test pulses.

Table I. Composition of solutions in millimoles per liter

	Bath		
	Solution 1	*Solution 2*	*Pipette—Solution 3*
Sodium chloride	120	60	5
Potassium chloride	5	5	120
Calcium chloride	3	3	0.1
TEA^+	0	60	—
HEPES	10	10	10
EGTA	–	–	1
pH	7.2	7.2	7.2

pH was adjusted to 7.2 ± 0.05 with sodium hydroxide for bathing solutions and with potassium hydroxide for the pipette solution. *EGTA*, Ethyleneglycol-*bis*-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid.

Current-voltage curves were obtained by plotting observed currents against test potentials. Membrane currents were measured from the holding level, and unless specifically noted, were not corrected for leak. Data for inactivation curves were obtained with the double-pulse technique—a varying conditioning potential that was immediately followed by a constant test potential. These currents were corrected for leak before analysis. Curve-fitting of the inactivation curves was performed with Marquardt nonlinear regression with the aid of STATGRAPHICS software (STSC, Inc., Rockville, Md.). The equation fit was the Hodgkin-Huxley formulation[8] for inactivation: $I_{in}/I_{in}(max) = (1 + \exp[(V - V_{0.5})/k])^{-1}$ Fig. 1 demonstrates the use of a voltage ramp to determine membrane capacitance. At the beginning of the tracing the holding potential is −100 mV. At time 0, a voltage ramp of 1.07 V/sec is begun and applied for 140 msec. Be-

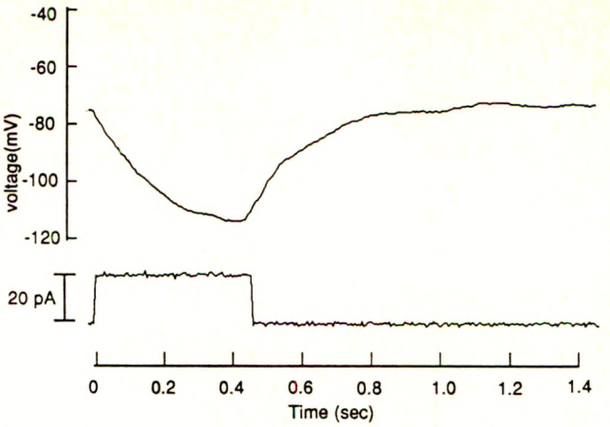


Fig. 2. Passive membrane potential response in the current clamp experiment. Bathing solution 1 (3 mmol/L calcium, 0 mmol/L TEA^+). A small hyperpolarizing current is applied to the cell to establish a resting membrane potential of −75 mV.

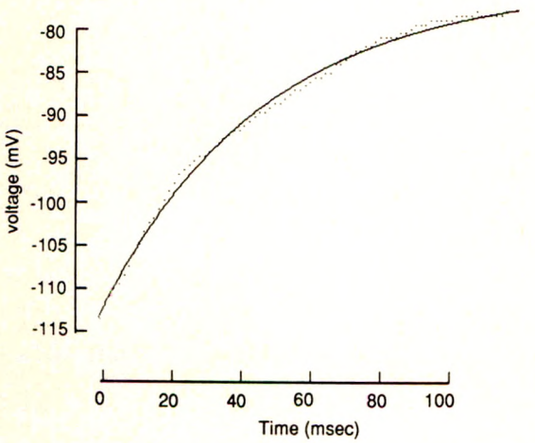


Fig. 3. Best-fit single exponential function *(solid line)* of voltage relaxation from data in Fig. 2.

tween −100 and −60 mV the response of the cell membrane is "passive" in that the electrical properties of the cell membrane can be described by a resistor (Rm) in parallel with a capacitor (Cm). The current response to the beginning of the voltage ramp is the charging of the membrane capacitance through the electrode resistance (Re) and is the current step that is seen at time 0 in Fig. 1. The capacitance is calculated from the height of the current step (Δi) through the relation $\Delta i = Cm \times \Delta V/\Delta t$, where $\Delta V/\Delta t$ is the ramp rate (1.07 V/sec). For the purpose of approximation of cell membrane surface area, we assume a unit membrane capacitance of 1.0 microfarads per square centimeter, which is found in other smooth muscle preparations,[9] and calculate the surface area from the capacitance measurement. Membrane resistances were calculated from the slope of the current response to the voltage ramp between −90 to −60 mV through the relation $Rm = \Delta E/\Delta i$. Unit membrane resistivities are membrane resistances that are corrected for cell surface area.

After the voltage-clamp experiments, current-clamp experiments were performed. Membrane potentials at zero applied current varied between −30 and −70 mV. Usually a small hyperpolarizing current was required to maintain the membrane potential in the −70 to −80 mV range.

Results

Passive membrane properties. Surface membrane capacitance and resistance measurements of freshly dispersed human uterine cells were obtained from voltage-ramp experiments (Fig. 1). The surface area of six cells varied between 8.0×10^{-5} and 2.9×10^{-4} cm^2. Measured membrane resistance values varied between 1 and 2 GΩ for those cells. Calculated resistivity varied between 0.8 and 2.25×10^5 Ω-cm^2.

Fig. 2 shows results from a current-clamp experiment and demonstrates membrane potential response to a hyperpolarizing current pulse. Fig. 3 shows the exponential fit of the relaxation phase of the membrane potential response that is shown in Fig. 2. This close fit is predicted by the equivalent circuit and indicates that the cell membrane properties are passive in this voltage range.

Membrane action potentials. Under conditions of 3mmol/L calcium, action potentials could be elicited with depolarizing current pulses or after a hyperpolarizing pulse (Fig. 4). The cell membrane voltage responses are pure expressions of membrane ion channel activity only under conditions in which the holding current is zero. Therefore analysis of action potentials was limited to those that were elicited by hyperpolarizing

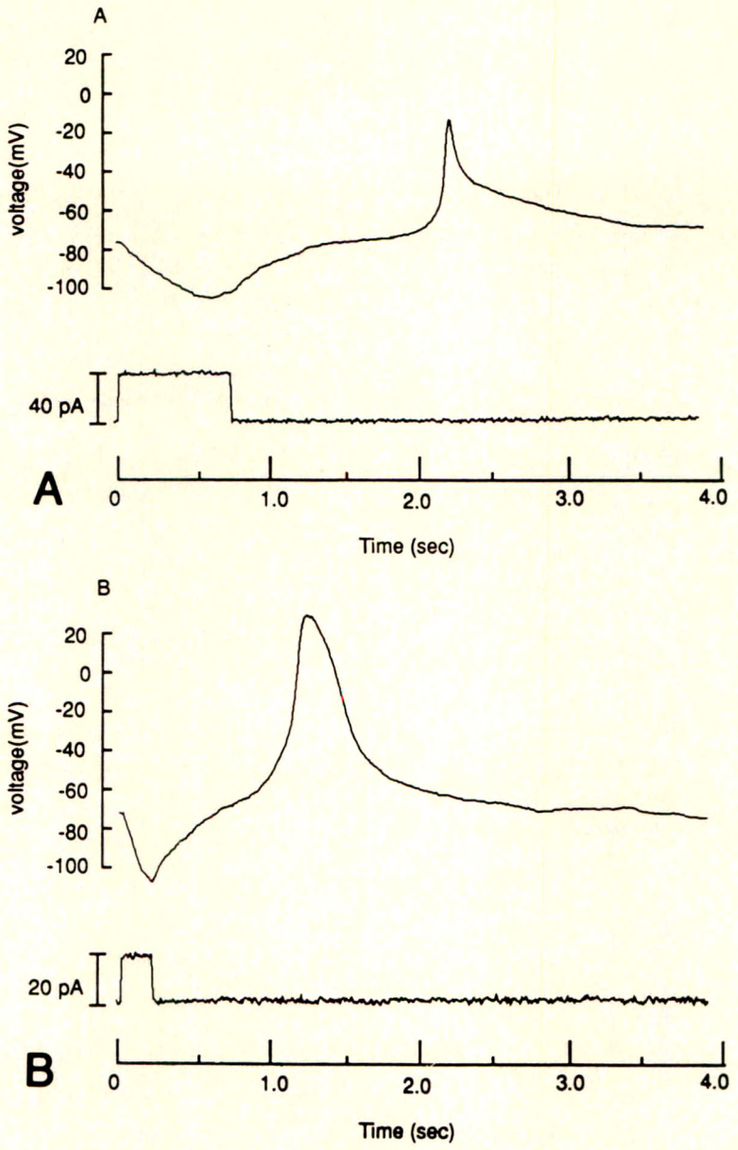


Fig. 4. Action potentials in the current-clamp experiment. **A,** Bathing solution 1 (3 mmol/L calcium, 0 mmol/L TEA^+). A 40 pA hyperpolarizing current pulse is applied for 750 msec. **B,** Same cell, with bathing solution changed to solution 2 (3 mmol/L calcium, 60 mmol/L TEA^+).

current pulses (anodal break excitation) or action potentials that occurred after depolarizing current pulses.

Without the use of blocking agents, the maximum depolarization that was observed in six cells was −17 mV (Fig. 4, *A*), and the maximal rate of voltage rise was 1.0 ± 0.2 V/sec for that cell. This voltage rise was the result of inward current that charged the membrane capacitance and is described by the relation $i = Cm \times dV/dt$. Thus, with the use of 1 $\mu F/cm^2$, the corresponding current density is 1.0 $\mu A/cm^2$.

With exchange of bathing solution so that TEA^+ (tetraethylammonium ion) (60 mmol/L) was substituted for sodium, the action potential duration lengthened, and the maximum depolarization increased to +20 mV (Fig. 4, *B*). However, the maximal rate of voltage rise for the action potential remained essentially unchanged at 0.79 V/sec. For three cells an average of 0.8 ± 0.2 V/sec was observed.

Action potential generation results from a complex interplay of ion channel opening and closing in response to membrane potential changes, calcium ion concentrations, and other parameters that affect membrane excitability properties. To help delineate the contribution of the calcium channel to the excitability properties of the membrane, whole-cell voltage clamp analysis was undertaken.

Voltage clamp analysis. In 3 mmol/L calcium under voltage-clamp conditions (Fig. 5), these cells demon-

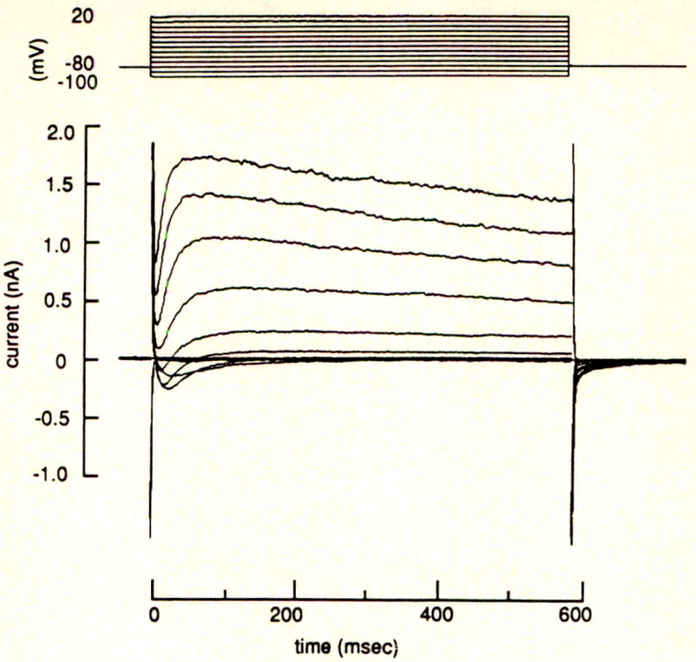


Fig. 5. Whole-cell voltage-clamp experiment. Current responses to stepped voltages. Bathing solution 1 (3 mmol/L calcium, 0 mmol/L TEA^+). Holding potential is −80 mV.

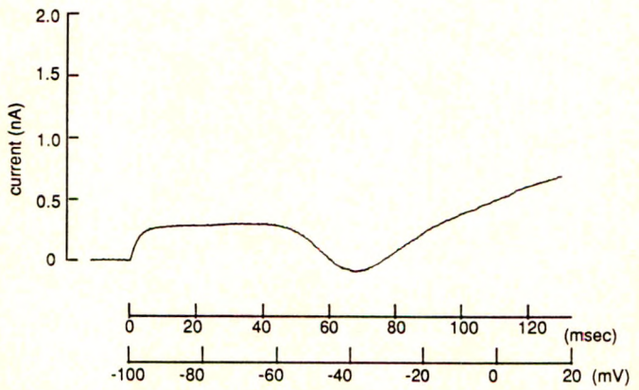


Fig. 6. Current responses to voltage ramp. Bathing solution 2 (3 mmol/L calcium, 60 mmol/L TEA^+). Data were obtained from same cell as in Fig. 1.

strated complex currents. Outward currents in excess of 1 nA dominated inward currents, which made quantitative analysis of inward currents difficult. To isolate the inward calcium currents for study, outward currents were reduced by the use of TEA^+ (60 mmol/L) in the bathing solution.[10, 11]

The effect of TEA^+ (60 mmol/L) on human uterine myocytes is most easily observed in the voltage-ramp experiment (compare Fig. 6 with Fig. 1). Between −60 and −20 mV, similar inward currents occur in both the absence (Fig. 1) and the presence (Fig. 6) of TEA^+. At potentials above −20 mV, outward current rises rapidly, as shown in Fig. 1. This outward current is inhibited by TEA^+ (Fig. 6) and is consistent with blocking of potassium channels by TEA^+.

Current responses of the cell to stepped voltages with the TEA^+ (60 mmol/L) bathing solution are shown in Fig. 7. With the reduction in outward currents, inward currents are more easily observed. The resulting current-voltage curve (Fig. 8) of the peak inward current demonstrates that activation of the inward current begins at −60 mV and maximal inward current occurs at −40 mV. The value of 275 pA for maximal inward current corresponds to a current density of 1.1 $\mu A/cm^2$ (measured membrane capacitance was 240 picofarads for this cell). This is in good agreement with

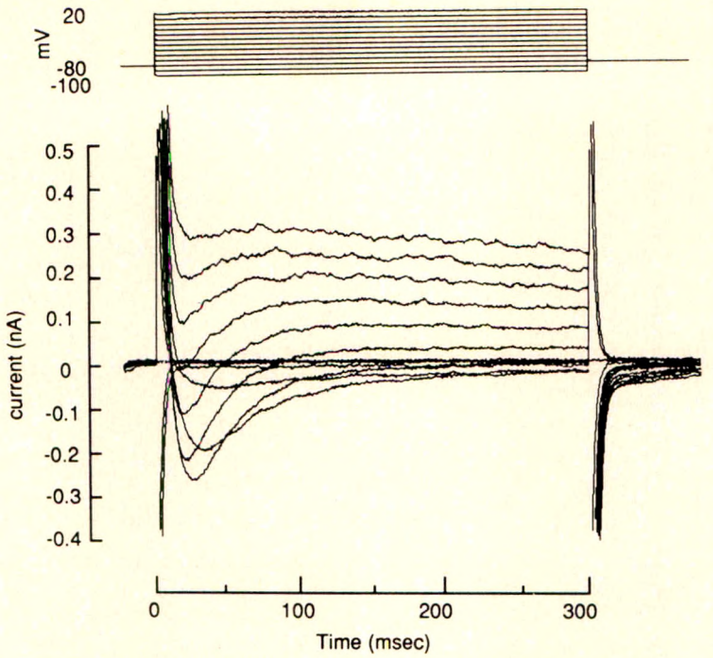


Fig. 7. Current responses to stepped voltages. Bathing solution 2 (3 mmol/L calcium, 60 mmol/L TEA^+). Holding potential and voltage protocol are identical to those in Fig. 5.

0.79 μA/cm², which was observed from the action potential of that cell.

Voltage inactivation of the inward current was performed by application of varying conditioning potentials for 250 msec followed immediately by a constant test pulse to −40 mV. A plot of the current responses to the test pulse versus conditioning potentials yields the Hodgkin-Huxley inactivation curve[8] (Fig. 9) with $V(1/2) = -54.9$ mV and $k = 4.5$ mV.

Comment

The experiments that are described here were performed on freshly dispersed pregnant human uterine smooth muscle cells. These cells readily formed gigaohm seals with patch pipettes and demonstrated reproducible passive and active properties under whole-cell voltage and current-clamp conditions. These experiments were performed on cells that were obtained from the lower uterine segment. Although the ideal tissue to study would be fundal tissue, those surgical specimens are difficult to obtain. Some elements of membrane excitability, such as channel density or receptor density, would be expected to vary depending on the site. However, the major features of membrane excitablity are the expression and regulation of ion-specific channels and are likely to be similar whether they originate in the fundus or the lower uterine segment.

Membrane resistivity. With the cell surface area as determined by capacitance measurement, membrane

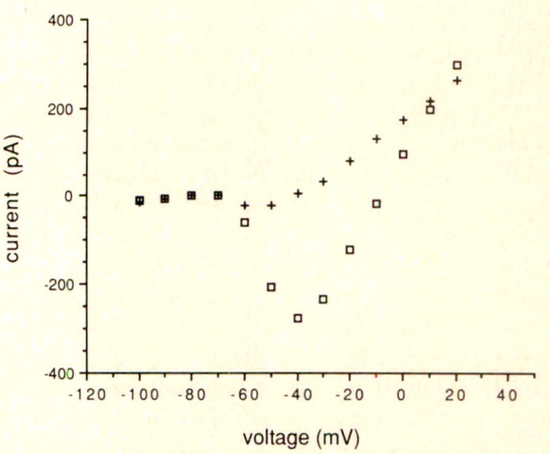


Fig. 8. Current-voltage relationship of data in Fig. 7 (3 mmol/L calcium, 60 mmol/L TEA^+). □, Peak inward current; +, current measured at 290 msec. The apparent reversal of inward current is artifact of decay of residual outward current.

resistivity in excess of 10^5 Ω-cm² in 3 mmol/L calcium was observed. The relatively large range among the six cells that are reported on here (from 0.8 to 2.25 × 10^5 Ω-cm²) is in part explained by difficulties in the determination of accurate measurements of very large resistances. Although pipette seal resistances were >5 GΩ, when 1 to 2 GΩ was measured, as much as a third of the current that was passed could be shunted by leakage of current from the electrode to the bath. Thus

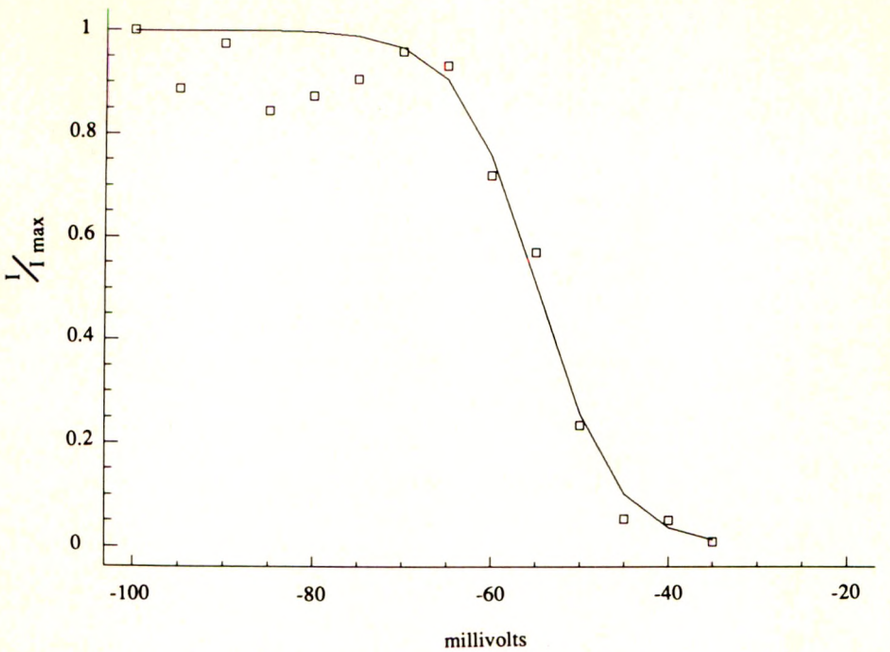


Fig. 9. Hodgkin-Huxley inactivation curve[8] of peak inward current. Data were obtained from same cell that was used to obtain data shown in Figs. 7 and 8. Best fit of data *(solid line)* yields $V_{1/2} = -54.5$ mV, K = 4.5 mV.

measured values of membrane resistivities should be interpreted as a lower limit. In addition, any significant cell damage during the isolation procedure would likely reduce measured membrane resistivity values.

These values are similar to those that have been reported for visceral[10, 12] and vascular[13, 14] smooth muscle cells. The importance of the unit membrane passive properties in relation to the active membrane properties, hormonal stimulation, site of biopsy, previous labor, or other clinical parameters awaits systematic study.

Inward current—the voltage-activated calcium channel. In agreement with many other smooth muscle systems, the inward currents that were observed in human myometrium are the expression of voltage-activated calcium channels.[1] The contribution of sodium currents[15] to the action potentials is probably not significant since replacement of half of the bathing solution sodium with TEA^+ increased rather than decreased the overshoot of the action potential (-17 to $+20$ mV). In addition, there was no significant change in the maximal rate of voltage rise of the action potential spike (1.0 vs 0.8 ± 0.2 V/sec). Further voltage-clamp studies in the 2 to 10 msec time frame will be required to determine whether sodium currents contribute to the action potential to any significant degree.

Evidence for two types of calcium channels was recently reported.[5] In this work, the voltage inactivation of the inward current (Fig. 8) was well fit by the Hodgkin-Huxley curve[8] with rapidly developing inactivation (k = 4.5 mV), which implies that the different types of calcium channels have similar, if not identical, inactivation characteristics.

In the cells that were studied in this experiment, there were only minor differences among the current-voltage relationships and the rate of decay of the inward current transients. Inward current was studied under ionic conditions that were as close to normal as possible. Specifically, a physiologic calcium concentration (3 mmol/L) was used to avoid artifactual changes of the current-voltage relationships that are noted in solutions with elevated calcium concentrations.[12]

Importance of the resting potential. The beginning of the inward current activation (-60 mV) is near the half-maximal inactivation (-54.9 mV). Thus this voltage range is crucial to the excitability of the cell membrane. The relatively short duration of the conditioning pulse (250 msec) in the inactivation protocol was chosen to ensure that inactivation of the calcium currents would occur on a time frame that was shorter than the time frame of slow-wave depolarization.[16] At resting membrane potentials that are more negative than -60 mV, no calcium ions flow into the cell, but calcium channels would be activated on slight depolarization. At resting potentials in which most of the calcium channels are inactivated (around -50 mV and more positive), no calcium ions flow into the cell and no action potentials would be generated regardless of further

depolarization. Thus a major controlling factor of the excitability of the membrane is the resting potential.

Propagation of the action potential through a syncytium. The ability of an intact uterus to undergo a coordinated contraction presumably requires propagation of the action potential from the fundus to the lower uterine segment. The propagation mechanism likely involves the action potential of one myocyte that causes depolarization of adjacent cells and leads to a spread of action potentials.[17] This would require myometrial cells that are at the leading edge of the action potential to be at a resting potential more negative than −60 mV; otherwise, propagation would cease. The presence of gap junctions[17] between adjacent cells allows for electrical communication and tends to equate the membrane potentials of the cells. With significant numbers of gap junctions, an electrical syncytium is established, within which all of cells are near the same resting membrane potential. Thus we believe that gap junctions not only aid in the transmission of the action potential from cell to cell but are required to establish membrane potentials along a path of propagation that is sufficiently negative to prevent calcium channel inactivation.

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Intraabdominal sound levels during vibroacoustic stimulation

Ernest M. Graham, MD,[a] Aemil J.M. Peters,[a] Robert M. Abrams, PhD,[a, b]
Kenneth J. Gerhardt, PhD,[c] and David J. Burchfield, MD[b]
Gainesville, Florida

Vibroacoustic stimulation is widely used in the United States as a test for fetal well-being, yet little information is available on the adequacy of the electronic artificial larynx that is commonly used as the stimulator. To determine whether the intraabdominal sound pressure level was dependent on the static force applied to the electronic artificial larynx, we measured the sound pressure level at a position 20 cm from the surface of the anterior abdominal wall in nonpregnant ewes. The electronic artificial larynx was pressed against the surface with three levels of static force: mild, moderate, and strong. Between mild and strong static forces there was a trend toward a reduction in sound pressure level at the fundamental frequency (85 Hz) and the overtones (170 to 1600 Hz) ($p < 0.08$). To further examine the relationship between the static force of sound source against the abdomen and the intraabdominal sound pressure level, sine wave oscillations between 20 Hz and 4.0 kHz were produced with an industrial shaker. With a constant dynamic force (0.16 N) applied to the shaker across frequencies, the sound pressure level was greatest at lower frequencies (100 to 110 dB) and less at higher frequencies, above 1.0 kHz (60 to 80 dB). Consistent with the electronic artificial larynx experiments, strong application of the shaker resulted in greater transmission of sound than did mild application ($p < 0.02$). (Am J Obstet Gynecol 1991;164: 1140-4.)

Key words: Vibroacoustic stimulation test, intraabdominal sound pressure level

The fetal vibroacoustic test has become a popular method for testing antepartum and intrapartum fetal health.[1-3] Although the test is useful in the prediction of fetal well-being when the stimulus produces accelerations in fetal heart rate and increased heart rate variability, the test is much less accurate in the prediction of poor outcomes.[4-7] The electronic artificial larynx, the device that is most commonly used for fetal vibroacoustic testing, produces exceedingly high intrauterine sound pressure levels, as judged by recent experiments in sheep,[8] but only in an area directly under the skin site to which it is applied. Movement of the electronic artificial larynx by just 3 inches reduces the sound pressure level by as much as 20 dB.[8] Thus the position of the stimulator is one important factor in the determination of the level of the stimulus that is detected by the fetus and, by inference, the fetus's response. Difficulties in locating the fetal head, for example, could reduce the likelihood of a positive response even in a healthy fetus.

When the electronic artificial larynx is held in one hand and pressed with considerable force into the palm of the other, one notices a change in timbre of the sound of the vibrating diaphragm, which suggests an electromechanical overload of the diaphragm. If one presses too lightly, it reduces the strength of the mechanical coupling between the electronic artificial larynx and the palm with a resultant reduction in energy flow into the hand. This effect has been noted in studies of whole-body vibration.[9] Thus the static force (nonoscillating, continuous force expressed in centimeters of skin depression) with which the electronic artificial larynx is applied to the abdominal skin may be another variable of sound transmission and resultant intrauterine sound pressure level and fetal response.

To determine the effects that the strength of mechanical coupling between the vibrator and the abdomen has on the transmissibility of sound pressure as a function of frequency, we applied an electronic artificial larynx and a shaker actuator, which were driven with sine waves from 20 Hz to 4.0 kHz, to the abdominal skin at different static forces; we then recorded the resulting intraabdominal sound pressure levels.

Material and methods

Guidelines for the care and use of the animals, which were approved by the University of Florida, were followed. Six nonpregnant ewes were anesthetized intravenously with pentobarbital and then killed with intracardiac potassium chloride. The stomachs and intestines were removed through a midline incision, flushed to remove any gas-forming bacteria, and replaced with adequate saline solution to expand the abdominal cavities to their former dimensions. The incisions were

From the Departments of Obstetrics and Gynecology,[a] Pediatrics,[b] and Communication Processes and Disorders,[c] University of Florida.
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Reprint requests: Robert M. Abrams, PhD, Department of Obstetrics and Gynecology, University of Florida College of Medicine, Box J-294, JHMHC, Gainesville, FL 32610.
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closed. A miniature hydrophone (model 8103, Bruel and Kjaer Instruments, Inc., Marlborough, Mass.), which was calibrated with a piston phone (model 4223, Bruel and Kjaer Instruments, Inc.), was placed intraabdominally through a stab wound in the flank of each ewe so that it was positioned 20 cm from the midline anterior wall.

Each ewe was placed on her side and vibration excitation was mechanically coupled horizontally against the midline skin by an electronic artificial larynx (Bell Telephone model 5-C, Western Electric, New York) or by a shaker (model PM 50, MB Electronics, Cleveland) controlled by a power amplifier (model 2250, MB Electronics). A schematic of the measurement setup with the shaker is shown in Fig. 1. An impedance head (model 8001, Bruel and Kjaer Instruments, Inc.) was placed in series with the actuator of the shaker. The dynamic force that was produced by the actuator was transduced by the impedance head, recorded as a root mean square voltage, and converted to newtons (kilograms × meters per square second). Dynamic force refers to that force that is produced by oscillation of a vibrator and calculated at the surface of the skin. Input force on the 1.32 cm^2 actuator head was held constant at 0.16 N for all test sinusoidal frequencies. The signals were produced by a frequency generator (model 3010, Synascan Corp., Chicago). The output of the force transducer was observed on an oscilloscope to verify the fidelity of the sine wave input to each animal.

Outputs from the hydrophone were conditioned with a charge amplifier (model 2635, Bruel and Kjaer Instruments, Inc.) and fed into a real-time frequency analyzer (model 2123, Bruel and Kjaer Instruments, Inc.). The analysis of the fundamental and harmonic contents of the signals throughout the frequency spectrum was achieved by measurement of constant percentage bandwidth filters. Spectra (1/24 octave; 1.4 Hz to 5.5 kHz) were averaged over 20 seconds, stored on a diskette, and plotted with a graphics plotter (model 2319, Bruel and Kjaer Instruments, Inc.). Spectral analysis results in a graphic display of the frequency and amplitude content of a particular stimulus.

Sound pressure at the fundamental frequency of the electronic artificial larynx and at all measurable overtones were recorded. In the shaker experiments we measured the sound pressure that was created by the sinusoidal input at frequencies of 20, 50, 75, 100, 150, 200, 300, 400, 500, 750 Hz and 1, 1.5, 2, 3 and 4 kHz.

The electronic artificial larynx and the shaker actuator were pressed against the animals' shaved skin to produce 1, 3, and 5 cm depressions. These depressions resulted in three degrees of static forces, which we termed: (A) mild, 1 cm depression of skin; (B) moderate, 3 cm depression; and (C) strong, 5 cm depression. The sound pressure level that was generated was compared across the frequencies by a two factor repeated measures analysis of variance, and the degree of vibrator force was used as a factor. The analysis was followed by a Tukey's multiple range test.

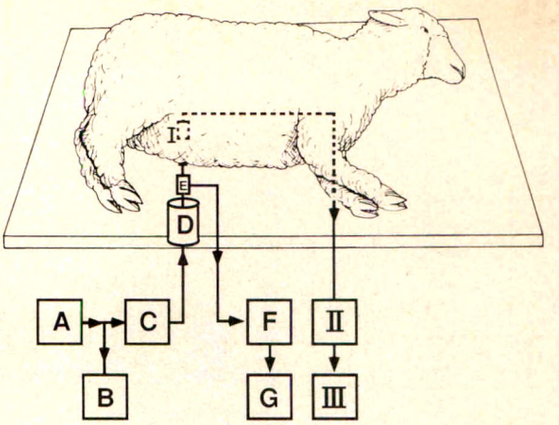


Fig. 1. Schematic drawing showing position of animal, location of hydrophone, and arrangement of devices for measurement of frequency, shaker force, and intraabdominal sound pressure levels (see text for explanation). *A*, Sine wave generator; *B*, frequency counter; *C*, amplifier; *D*, shaker; *E*, force transducer; *F*, preamplifier; *G*, root mean square voltmeter; *I*, hydrophone; *II*, charge amplifier; *III*, real-time frequency analyzer.

Results

Spectral analyses of signals from the intraabdominal hydrophone during stimulation with an electronic artificial larynx revealed a clear fundamental frequency of 85 Hz and many overtones (Fig. 2). From this figure it becomes apparent that the frequencies below approximately 1.0 kHz are in excess of the noise floor by 20 to 30 dB, whereas the levels at higher frequencies tend to be indistinguishable from the noise floor.

Results of the average amplitudes of the fundamental frequencies and overtones for the three conditions of static force are included in Fig. 3, along with the noise floor. The noise floor *(inverted triangles)* was determined by evaluation of the levels in 1/24-octave bands at frequencies immediately adjacent to the fundamental frequencies and overtones. As a group, sound pressure levels that were created by mild static force appeared to be lower than sound pressure levels that were produced by strong depression ($p < 0.08$) (Fig. 3). The disparity among sound pressure levels that were produced by varying electronic artificial larynx depression was greatest for frequencies above 1.0 kHz. With the electronic artificial larynx applied by mild force to the abdominal wall, the sound pressure levels above 1.0 kHz blended into the noise floor, whereas medium and strong static forces led to clear sound pressure levels that were above this background noise. Thus the greater the strength of application of the electronic

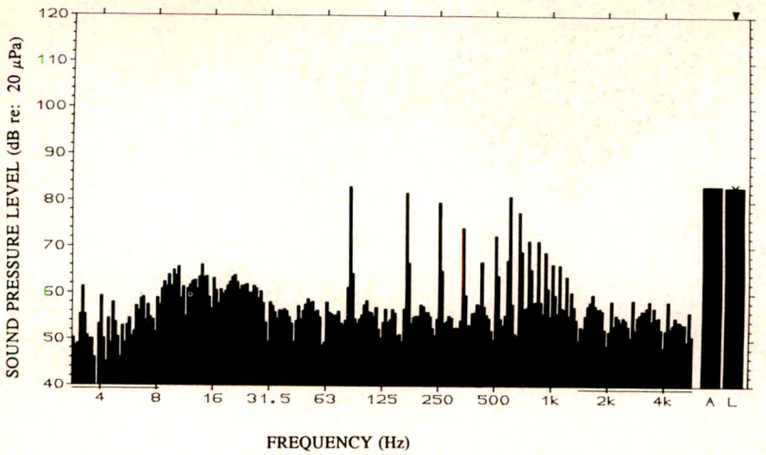


Fig. 2. 1/24-Octave band spectral display of intraabdominal sound pressure during stimulation with an electronic artificial larynx, which was applied with moderate static force.

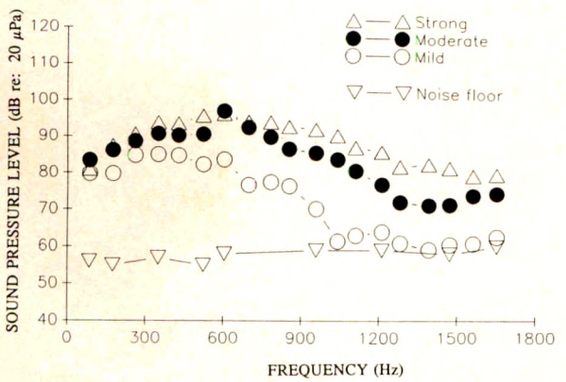


Fig. 3. Mean intraabdominal sound pressure ($n = 6$) recorded in 1/24-octave bands for the fundamental frequencies and overtones produced by an electronic artificial larynx applied to abdominal skin. Three static forces were used during stimulation with the electronic artificial larynx: ○, mild (1 cm depression of skin); ●, moderate (3 cm depression); and △, strong (5 cm depression). *Inverted triangles* represent average sound pressure in 1/24-octave bands that are immediately adjacent to fundamental frequencies and overtones and are representative of the noise floor for animals and recording equipment.

artificial larynx, the more likely it is that higher frequencies will penetrate to the hydrophone.

Similar results were found with the use of pure tones at selected frequencies that were generated by the shaker. Fig. 4 shows the two spectra that were produced in one animal with moderate static force of the shaker actuator against the skin and a constant dynamic force of 0.16 N. The 75 Hz pure tone (A) produced a signal over 100 dB, which is 40 dB above the noise floor, whereas a 750 Hz tone produced a signal of about 80 dB, which is only 20 dB above the noise floor. For the group as a whole, mild application of the shaker actuator led to lower sound pressure levels, as detected by the hydrophone than with strong application ($p < 0.02$) (Fig. 5).

Comment

The electronic artificial larynx is a fixed amplitude device that produces vibroacoustic energy over a wide spectral range.[8, 10] It was designed to aid aphonic patients in the generation of audible speech. Slight variations in force and position of the electronic artificial larynx against the throat modify the intensity and timbre of the sounds that are resonated by the nasopharyngeal and oral cavities. Coupling between vibrator and tissue is also important in the obstetric evaluation. With higher wall tensions, as might be expected in the tightly stretched abdomen of a slender pregnant woman at term, coupling would be expected to be optimal with less static force from the electronic artificial larynx.

At frequencies between 600 and 4000 Hz, the effect of skin depression and thus coupling between vibrators and abdominal contents was best observed. Interpretation of the results of the present experiments is hindered by the fact that the diminished distance between vibrator and hydrophone during strong depression (5 cm) of the vibrator may also have contributed to the higher sound pressure levels. As previously shown by Gerhardt et al.,[8] movement of an electronic artificial larynx laterally from its position immediately over a hydrophone leads to dramatic alterations in recorded intrauterine sound pressure level. In the current study at frequencies of 500 Hz and below, sound pressure level was less dependent on the depression-related coupling between electronic artificial larynx and the abdominal contents. Unless the electronic artificial

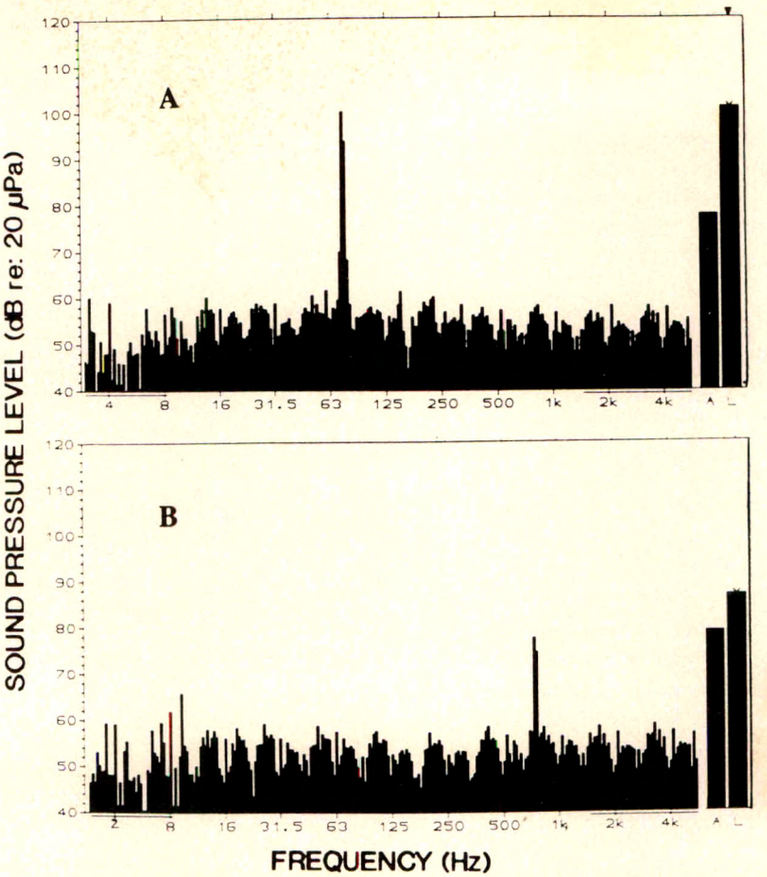


Fig. 4. 1/24-Octave band spectral displays of intraabdominal sound pressure during sinusoidal vibration of abdominal close-up surface at 75 Hz **(A)** and 750 Hz **(B).** Moderate static force was applied to the actuator.

larynx is pressed strongly against the abdomen, the sound pressure that is produced by vibration may not exceed the noise floor at frequencies of 1.0 kHz and above. Considering that one can expect an additional 10 to 15 dB of internal noise to be generated by the live, semirestrained animal,[11] it may be difficult to produce a high-frequency signal that can be detected by the fetal ear at some distance from the skin surface, even with strong depression of the electronic artificial larynx. Correct identification of the position of the fetal head before the vibroacoustic stimulation test is thus clearly required.

The noise floor of 50 to 60 dB (1/24-octave bands) represents the combined effects of room vibrations and sounds, tremors of the hand that is holding the electronic artificial larynx, and the hydrophone and charge amplifier noise floor. In comparison to the sound pressure level, which is created across many frequencies by the electronic artificial larynx, the signals that are produced by pure-tone sine waves can be made much more intense. By increasing the electrical power to the shaker,

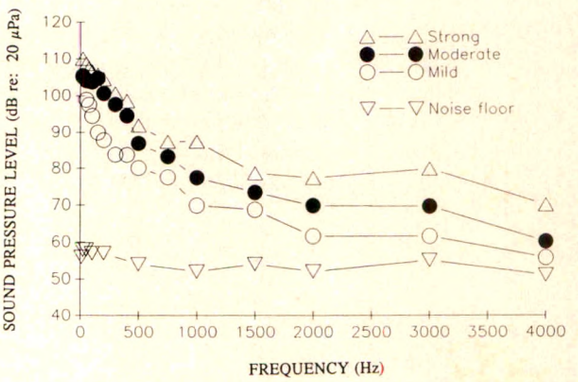


Fig. 5. Intraabdominal sound pressure recorded in 1/24-octave bands during application of a shaker with a constant dynamic force on the skin surface at several frequencies between 20 Hz and 4 kHz. Three static forces were used during sinusoidal stimulation: ○, mild (1 cm depression of skin); ●, moderate (3 cm depression); and △, strong (5 cm depression). *Inverted triangles* represent average sound pressure in 1/24-octave bands that are immediately adjacent to fundamental frequencies and overtones and are representative of the noise floor for animals and recording equipment.

much of the pressure can be focused in a single narrow band.

However, it is not known whether single pure tones are more efficacious than broader band signals in eliciting a fetal response. Gagnon et al.[12] reported an immediate and sustained increase in long-term fetal heart rate and the number of fetal heart rate accelerations when human fetuses were stimulated with a square wave at 100 Hz. An earlier study with a 2.0 kHz signal, which was produced by an earphone, showed increases in fetal movement after 1 minute of acoustic stimulus to the pregnant woman.[13] Although our study was not intended to evaluate fetal response, it is clear that low-frequency pure tones penetrate the abdomen with virtually no attenuation as a function of distance.

For both broad-spectrum sounds that are produced by an electronic artificial larynx and pure tones that are produced by a shaker, sounds at lower frequencies are transmitted unaffected by the static force that is applied to the abdomen, whereas signals at higher frequencies are absorbed. This is because the extent to which vibroacoustic energy penetrates tissue is dependent on frequency. Low frequencies with long wavelengths resemble acoustic compression waves and pass through tissue with little loss of energy. High frequencies with short wavelengths result in damped shear waves, and consequently, the energy is dissipated near the surface. The depth of penetration varies as the reciprocal of the square root of the frequency.[14]

Although the present experiments with dead, nonpregnant sheep might not totally simulate the conditions of supine, pregnant women at term, the results should alert obstetricians to two of the factors—mechanical coupling and vibration frequency—which must be considered in short-term diagnostic vibration exposures. Also of concern are these factors in exposures of longer duration of vibration, which are often encountered in the workplace. Any effect of vibration on the fetus is likely to be determined by a host of other factors including the mass and resonant frequencies of the abdominal segment, the thickness of the tissue that intervenes between maternal skin and fetus, the surface area of vibrating device, the vibration vector, and age of the fetus.

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Effect of progesterone on renin secretion in endometrial stromal, chorionic trophoblast, and mesenchymal monolayer cultures

Dinesh M. Shah, MD,[a, b] Kazumi Higuchi, MD, PhD,[c] Tadashi Inagami, PhD,[c] and Kevin G. Osteen, PhD[b]

San Antonio, Texas, and Nashville, Tennessee

Renin messenger ribonucleic acid expression has been shown in decidual tissue and endometrium, but weakly in chorion laeve, suggesting decidua as a primary source of uteroplacental renin. We proposed to confirm active renin secretion by endometrial stromal cells and examined the effect of progesterone-induced decidualization on secretion. Separate monolayer cultures of endometrial stroma, trophoblast, and mesenchymal cells were established and maintained for 10 to 15 days. Active renin concentration was measured with radioimmunoassay for angiotensin I generation and expressed as picograms per milliliter of angiotensin I generation per 10^5 cells. Active renin concentration was high in control secretory endometrial stromal cells (513 pg/ml) compared with trophoblast (none) and mesenchymal cell cultures (74 pg/ml). Marked decrease in active renin secretion occurred in control endometrial stromal cells (days 13 to 15, 86 pg/ml), whereas progesterone-induced decidualization sustained and increased secretion (days 13 to 15, 1017 pg/ml). This renin activity was quantitatively inhibited in culture fluid assays by specific human renal renin antibody in serial dilutions. Renin activity measurements before and after trypsin activation showed the majority of renin (91.15%) in the inactive form and a smaller fraction (8.85%) in the active form. Immunohistochemistry with the use of specific human renal renin antibody confirmed the presence of renin and its stimulation by progesterone in endometrial stromal cells. Progesterone had minimal effect on mesenchymal cells. These data confirmed endometrial stromal cells as a significant source of active renin and showed that progesterone induced a marked increase in this production. (Am J Obstet Gynecol 1991;164:1145-50.)

Key words: Renin in reproductive tissue cultures, hypertension in pregnancy

The renin-angiotensin system is recognized to have an important physiologic role in pregnancy. Brown et al.[1] found the plasma renin concentration in pregnant women to be greater than that in nonpregnant women. Although a significant part of this increased renin is present in its inactive form, plasma concentration of active renin is also increased in pregnancy.[2] It has been difficult to ascribe a role to renin in hypertensive disorders of pregnancy. Tapia et al.[3] found no significant difference in plasma renin activity and plasma renin substrate between normotensive and hypertensive pregnant women. Weir et al.[4] found plasma renin concentration, plasma renin substrate, angiogensin II, and aldosterone levels to be lower in the hypertensive group as compared with normal pregnancy.

The uteroplacental unit has been evaluated for its contribution to the renin-angiotensin system in pregnancy. Broughton-Pipkin et al.[5] found plasma renin concentration in the uterine vein to be no different from that in the antecubital vein in normotensive pregnancy, but in hypertensive subjects plasma renin concentration was significantly suppressed in the antecubital vein, as compared with the uterine venous level in the same subjects. The uteroplacental unit is also recognized to be a source of elevated circulating prorenin levels in normal pregnancy.[6] A role for the reproductive renin angiotensin system in the modulation of uteroplacental blood flow has been investigated. Gorden et al.[7] found the rabbit uterus to be a site of renin synthesis. Ferris et al.[8] have shown an increase in rabbit uterine renin secretion after experimentally induced uterine ischemia and suggested a role in the regulation of uterine blood flow.

Various tissues of the genital tract and fetoplacental unit have been shown to produce renin. Renin production by in vitro cultures of human chorion and uterine muscle was reported by Symonds et al.[9] Localization

From the Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio,[a] and the Departments of Obstetrics and Gynecology[b] and Biochemistry,[c] Vanderbilt University School of Medicine.

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Reprint requests: Dinesh M. Shah, MD, Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284-7836.

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of renin in trophoblasts in human chorion laeve at term pregnancy was shown by Poisner et al.[10] with immunohistochemistry. In an attempt to define the specific tissue responsible for reproductive renin, Shaw et al.[11] examined renin messenger ribonucleic acid expression in various reproductive tissues and suggested that decidua is a primary source of uteroplacental renin production. Recently, Symonds[12] reviewed the subject of renin-angiotensin system in pregnancy and defined four issues that need to be addressed when the role of renin in reproduction is considered: (1) the site of renin synthesis; (2) the nature of extrarenal renin; (3) the mechanism regulating renin release; and (4) the role of the system in the genital tract. The purpose of this investigation was to confirm endometrium as the primary tissue responsible for reproductive renin secretion in a tissue culture system, to examine the nature of this renin, and to examine the role of progesterone-induced decidualization in endometrial renin secretion.

Material and methods

Monolayer cultures

Endometrial specimens. Biopsy specimens were obtained in the secretory phase of the menstrual cycle in premenopausal women undergoing clinical evaluation. Isolation and culture of a highly purified population of stromal cells were established by a previously described method.[13] Briefly, biopsy specimens were placed in an ice-cold 1:1 mixture of Dulbecco's modified Eagle's medium/nutrient mixture F-12, dissected into small pieces, and washed by centrifugation. Enzymatic dissociation of tissue was accomplished in two distinct steps with 0.5% collagenase, 0.02% deoxyribonuclease, and 2% chicken serum. An intervening series of filtrations was done sequentialy through two monofilament nylon mesh membrane filters of 105 μm and 88 μm, respectively. The filtered stromal-enriched fraction was washed twice in Dulbecco's modified Eagle's medium/nutrient mixture F-12 and separated further from epithelial cell clumps by differential sedimentation at unit gravity. Last, the stromal cells from the second sedimentation were filtered through a 37-μm nylon mesh filter for final purification. After an initial 2-day incubation, the cells were cultured up to 15 days in serum-free Dulbecco's modified Eagle's medium/nutrient mixture F-12 with insulin, transferrin, sodium selenite media supplement in 24-well tissue culture plates at 37° C in an atmosphere of 95% air and 5% carbon dioxide. Paired samples of endometrial stroma were incubated with and without progesterone. Progesterone (50 ng/ml) was added to culture media for progesterone-induced decidualization.[14] Culture media were changed and collected at 3- to 4-day intervals and kept frozen at −20° C until radioimmunoassay for renin.

Chorionic cultures. Chorionic villus samples were obtained between 9 to 11 weeks' gestation for clinical indications. Larger pieces of villous material were separated from maternal decidua and blood clots with fine-point needles and forceps under sterile conditions. Villi were washed with 20% Chang medium and 80% Dulbecco's modified Eagle's medium mixed with 20% fetal calf serum. Enzymatic dissociation of trophoblast was carried out with 0.25% trypsin and mild agitation with a sterile pipette. Supernatant with trophoblast cells was removed from the mesenchymal sediment and centrifuged to remove trypsin. The cell pellet was plated in a flask that contained Dulbecco's modified Eagle's medium/nutrient mixture F-10 and Chang medium and incubated at 37° C in 5% carbon dioxide. Mesenchymal core sediment was treated with collagenase (50 mg in 7 ml of medium without fetal bovine serum), incubated for 2 hours, centrifuged, and then the cell pellet was plated in a flask in Dulbecco's modified Eagle's medium/nutrient mixture F-10 and 20% Chang medium and incubated at 37° C in 5% carbon dioxide. Culture fluids were changed and collected at intervals of every 3 to 5 days and kept frozen at −20° C until radioimmunoassay for renin.

Collection of these tissues was approved by the Committee for Protection of Human Subjects of Vanderbilt University Medical Center. Microscopic examination of these primary cultures showed that endometrial stromal cells were an elongated spindle shape with no obvious cell-to-cell processes. Trophoblast cells had epithelial-like morphology with multinucleated syncytia formation and mesenchymal cells were short with a spindle shape and small nuclei. Furthermore, it revealed that these samples contained more than 99% morphologically pure cell populations. Purity of endometrial stromal cell cultures has also been previously established.[13]

Radioimmunoassay for renin concentration. Renin concentration was measured in 50 μl of culture fluid incubated with sheep substrate for 60 minutes for angiotensin I generation. Radioimmunoassay of angiotensin I was peformed after a minimum 16-hour reaction time at 4° C with a previously described method.[15]

Trypsin activation of prorenin. Trypsin activation of inactive renin was carried out in 10 μl of sample with trypsin solution in the presence of bovine serum albumin over 30 minutes at room temperature. Reaction was halted with soybean trypsin inhibitor. Radioimmunoassay for angiotensin I was performed as described in this article to measure total renin. Trypsin-activable renin was calculated by subtracting active renin from total renin measurements.

Immunohistochemistry. After hydrogen peroxide and methanol treatment, washed, fixed, cultured

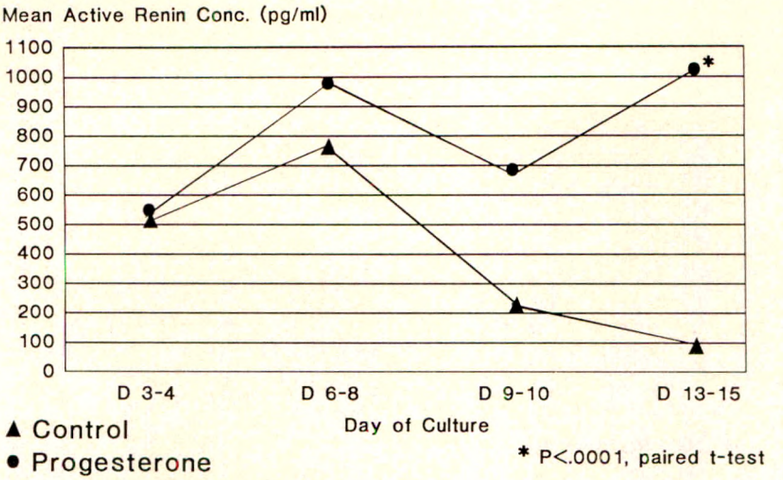


Fig. 1. Active renin concentration (picograms per milliliter angiotensin I generation per 10^5 cells) in endometrial stromal cultures and effect of progesterone-induced decidualization.

Table I. Active renin concentration (picograms per milliliter angiotensin generation per 10^5 cells) in control and progesterone-induced decidualized endometrial stromal cultures

	Day of culture							
	3-4		*6-8*		*9-10*		*13-15*	
	Control	p	*Control*	p	*Control*	p	*Control*	p
ECB 70	810	598	133	402	73	471	32	1012
ECB 75	667	785	45	1118	6	913	0	827
ECB 72	—	—	2729	2146	673	1299	299	1203
ECB 74	63	240	136	244	164	17	13	1026
Mean ± SEM	513 ± 206	541 ± 160	761 ± 656	978 ± 433	229 ± 152	675 ± 277	86* ± 71	1017* ± 77

*$p < 0.0001$, the paired Student t test.

cells were treated with specific rabbit antihuman renal renin antibody (1:500 dilution). The specificity of this antibody had been characterized.[16] Horseradish peroxidase-streptavidin conjugate, combined with biotinylated secondary antibody (goat antirabbit immunoglobulin G) and nickel chloride–3,3′-diaminobenzidine was used as a visual marker. Counterstaining with eosin B was used to enhance contrast between renin-positive sites and background.

Statistics. Statistical comparison for control and progesterone-induced decidualized endometrial stromal cell renin produciton was made by the paired Student t test.

Results

Four specimens of secretory endometrial stromal cells, two of trophoblast, and two of mesenchymal core were studied for renin secretion. Table I and Fig. 1 show active renin concentration in endometrial stromal culture fluids, measured as angiotensin I generation over 60 minutes. Cell counts were performed by trypsinization, dilute (0.08%) trypan blue staining, and counting live cells in a hemocytometer chamber. Renin secretion is expressed as picograms per milliliter per 10^5 cells for paired specimens of control and progesterone-induced decidualized cells. There is a significantly high (1017 pg/ml vs 86 pg/ml, $p < 0.0001$, paired Student's t test) active renin concentration in culture fluid of progesterone-treated cells compared with control cells on days 13 to 15. Trophoblast culture fluids assayed simultaneously failed to reveal any renin activity. Mesenchymal cell culture fluids showed a small amount of active renin concentration (range, 25 to 114 pg/ml). This is shown in Table II and Fig. 2.

Renin specificity and trypsin activation. Culture fluid specimens obtained on 5 different days from two additional cultures of endometrium were studied for inhibition of renin activity by renin antibody and trypsin activation of renin. Quantitative inhibition of this renin activity was obtained with serial dilutions of specific human renal renin antibody (Fig. 3). Results of trypsin activation are presented in Table III. Approx-

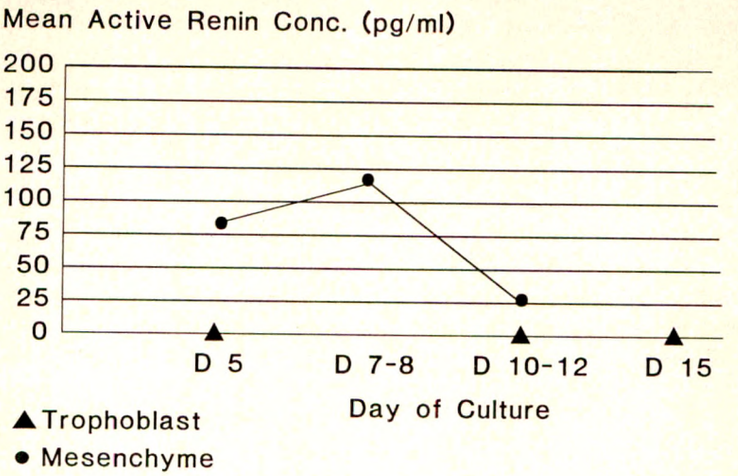


Fig. 2. Active renin in trophoblast and mesenchymal culture fluids expressed as picograms per milliliter angiotensin I generation per 10^5 cells.

Table II. Active renin concentration (picograms per milliliter angiotensin generation per 10^5 cells) in mesenchymal cell culture

	Day of culture		
Sample	*Day 5*	*Day 7-8*	*Day 12*
J.O.	95	103	25
M.G.	70	125	—
Mean ± SEM	82.5 ± 12.6	114 ± 11	25

Table III. Active and trypsin activated renin concentration in endometrial stromal cell cultures (nanograms per milliliter angiotensin generation)

Specimen	*Active*	*%**	*Trypsin-activated*	*%†*
A	33.72	23	133.55	77
B	3.04	5.5	52.57	94.5
C	4.42	5.2	81.45	94.8
D	1.32	6.2	20.11	93.8
E	0.25	4.2	5.85	95.8
F	1.78	9	18.09	91

*Average, 8.85%.
†Average, 91.15%.

imately 9% renin was in the active form and 91% in the inactive form.

Immunohistochemistry. Progesterone-induced decidualized endometrial stromal cells showed strongly positive staining by specific human renal renin antibody as compared with control cells, which showed only diffuse staining (Fig. 4). Mesenchymal cells showed minimal staining with a slight increase after progesterone treatment.

Comment

Renin activity has been noted to increase in pregnancy. Therefore the renin-angiotensin system has long been suspected to play a role in reproduction. It has been difficult to ascribe a role for renin in hypertension of pregnancy. Study of the renin-angiotensin system in uteroplacental blood has suggested it may play a role in uteroplacental blood flow regulation. The rabbit uterus has been shown to produce renin,[7] and increased renin secretion after experimentally induced ischemia in rabbits[8] suggests a role for renin in the regulation of uterine blood flow. In the chronically instrumented pregnant sheep, the effect of angiotensin II infusion[17] is reported to be related to the dose of infused angiotensin II. Increase in uterine blood flow occurred at low-dose infusion and a decrease was noted at high-dose angiotensin II infusion. This was explained[17] because increased perfusion pressure at low-doses offsets the effect of modest increases in uterine vascular resistance; whereas at high doses, a marked increase in uterine vascular resistance resulted in a decrease in uterine blood flow. This suggests that renin-angiotensin system may have a role in uterine vascular constriction and in the pathophysiology of uterine ischemia.

Renin production has been shown by a number of tissues of the human reproductive tract. Some of the previous studies have focused on identification of chorionic renin. In a study by Poisner et al.[10] renin was shown in chorionic membrane and in isolated cells of chorionic membrane by indirect immunofluorescence with rabbit antihuman renal renin antibody. It is conceivable that some of the immunofluorescence in the membrane may be in the mesenchymal cells. It is important that the method used for preparation of isolated cells with collagenase in that study may, at best, have provided a mixed preparation of trophoblast and mesenchymal cells. Our study with essentially pure cell

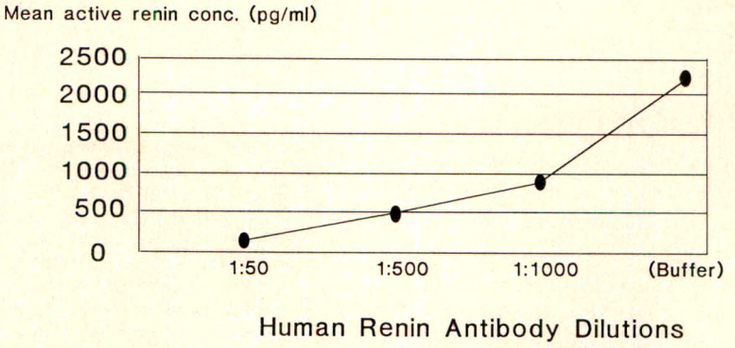


Fig. 3. Quantitative inhibition of renin activity by specific human renal renin antibody.

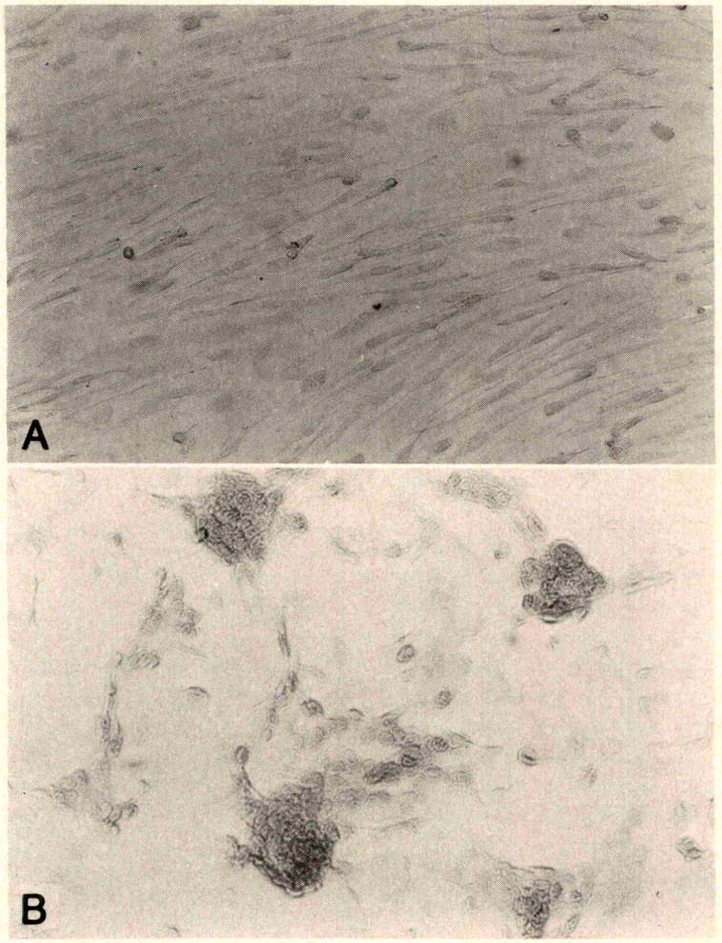


Fig. 4. Immunohistochemical evidence of renin in endometrial stromal cells in culture. **A,** Control cells; note spindle-shaped endometrial stromal cells with no staining by renin antibody. **B,** Progesterone-treated cells show morphologic features of in vitro decidualization. Note areas of enlarged cells with markedly dark staining in the cytoplasm by renin antibody.

populations failed to reveal any renin secretion by the trophoblast cell cultures and showed only a very small amount by the mesenchymal cell cultures under these experimental conditions. Thus our results are in disagreement with a previous study[10] that suggested the presence of chorionic renin of trophoblastic origin, but confirms secretion in small amounts by chorionic mesenchymal cells. Recent studies[11] of renin messenger ribonucleic acid have suggested endometrial cells, specifically decidua, to be the primary source of repro-

ductive renin. There is concern with regard to the nature of reproductive renin, inasmuch as a large portion of this tends to be inactive renin.[6]

Our experiments examined renin secretion in endometrial culture fluids specifically for active and trypsin-activable renin. We used a recently described method to obtain pure endometrial stromal cell cultures.[13] We experimentally induced decidualization with progesterone.[14] Significant renin secretion was found in endometrial stromal cells isolated from secretory endometrium. There was marked decrease (from 513 pg/ml to 86 pg/ml) in active renin secretion when cultures were maintained over a 15-day period. This decrease in renin secretion was prevented by progesterone treatment in paired samples and a marked increase (from 541 pg/ml to 1017 pg/ml) in active renin secretion was noted by days 13 to 15. This renin activity was quantitatively inhibited by specific human renal renin antibody. This suggests that renin-like material in the endometrial culture fluid is immunologically identical to purified human renal renin. This is important because other proteases (e.g., cathepsin D) have been shown to behave enzymologically similar to renin.[18] When renin acitivty was measured with and without trypsin activation, most of the renin (~91%) was present in the inactive form. This has also been the experience in other types of cells in culture.[19, 20] These culture fluid data were further substantiated by immunohistochemistry results, suggesting that endometrial renin secretion is influenced in paracrine fashion by progesterone. Because increased renin in peripheral blood has been noted in the secretory phase of the menstrual cycle[21, 22] and a significant increase in peripheral plasma renin concentration occurs in the first trimester of pregnancy,[2] our data further substantiate the concept of reproductive renin being under progesterone control. A significant aspect of our study is that a portion of this renin (~10%) is in the active form. This then may have a role in vasomotor modulation of uterine vasculature in pregnancy. Further studies are needed to establish this progesterone-induced renin secretion in experimental cultures and to evaluate its role in the regulation of uteroplacental blood flow.

We gratefully acknowledge the technical support of Laura Hobbs, BS, Mary Stevenson, MS, and Lynne Black, BS.

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LETTERS TO THE EDITORS

A method for safer urinary incontinence surgery

To the Editors: I would like to share a technique that I have now used successfully for the past 3 months. This past summer, while I was teaching a resident to do a Burch urethropexy,[1] I realized the inherent danger that is involved in directing a needle toward the "unprotected" finger in the vagina. After some thought, I purchased a packet of metal sewing thimbles for the reasonable price of 65 cents. Our nurses autoclave these for me.

With the proper size thimble placed over the surgeon's gloved index finger, the stitches can be placed safely and comfortably. The middle finger is left "bare" to provide better tactile sensation when the surgeon palpates the urethra. I have also used this technique in Richardson repairs[2] and it worked well. A little practice may be necessary to get comfortable with the use of the thimble Surgeons can practice with a thimble, a needle holder, a needle and suture, and a surgery towel to simulate the vaginal tissue. Also, use of the thimble will probably improve the tissue purchase a surgeon takes. As an experiment, compare the size of the "bite" in the surgical towel taken with the thimble as opposed to that taken with the "unprotected" finger. Both the Burch urethropexy and the Richardson repair are excellent procedures, and I have often thought that the reason for the occasional failures is inadequate suture placement. Although I have not tried it, the thimble should also work nicely in Marshall-Marchetti-Krantz procedures.

I heard this week that Dr. David Richardson, who has contributed greatly to the field of urogynecology, has designed a device that serves a similar purpose. At present, the sewing thimble is more universally available and less expensive.

I may not be the first person to think of this and certainly would not want to take undeserved credit. However, after I talked to some of the experts in the field of urogynecology, I found that none had tried this method. I am presently accumulating data on my "thimble" patients, and thus far all of them have done well.

Today, all of us must treat each patient as a potential source of infection and take precautions. I am compelled to share this technique with my colleagues with the hope that it will protect them from needle sticks, improve their surgical results, and make teaching these procedures safer and easier.

John W. Gallaspy, MD

Department of Obstetrics and Gynecology, E.A. Conway Memorial Hospital, P.O. Box 1881, Monroe, LA 71210-1881

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Fetal distress

To the Editors: We read with interest the article on fetal distress by Parer and Livingston (Parer JT, Livingston EG. What is fetal distress? AM J OBSTET GYNECOL 1990;162:1421-7). Their hypothesis that the onset of decompensation of the fetal physiologic responses to asphyxia is heralded by the occurrence of a decrease or loss of fetal heart rate (FHR) variability in the presence of stressed FHR patterns is an interesting one.

In our current quest for more objective indicators of clinically significant fetal asphyxia occurring during labor, we need to define clearly and evaluate prospectively those fetal physiologic functions that closely mirror the time course, the degree, and the duration of the asphyxial insult. Several fetal hemodynamic, biochemical, and electrophysiologic functions lend themselves to this purpose,[1] and fetal electrocardiogram (ECG) is one of them. Until recently, the wealth of potentially useful information conveyed by fetal ECG could not be tapped because of a variety of problems. With the arrival of newer generation of microprocessors and digital computer systems based on these microprocessors, the twin major hurdles of noisy fetal ECG signal and inability to handle and process voluminous digital data in real time have been overcome by the use of suitable software techniques.[2]

As a result of these developments in the field of digital electronics, it is now possible to obtain from the fetal ECG signal, besides FHR and its variability, information on time intervals (e.g., PR interval, QRS duration) and ST segment position continuously during labor. In a clinical study of fetal ECG with a minicomputer system equipped with time coherent averaging software routine, Murray[3] found that in the presence of a positive correlation between FHR and PR interval significant changes in the position of ST segment indicated increasing acidity of the fetal blood. He also reported that the on-line monitoring of the relationship between FHR and PR interval allowed the clinician to distinguish autonomically mediated FHR decelerations (with increased PR interval) from those that were metabolically mediated (i.e., a result of fetal hypoxia). Additional reports of the benefits of monitoring fetal ECG and the different methods used to monitor this signal may be found in a recent publication.[4]

Taken in conjunction with FHR variability, the additional information provided by the time intervals and the position of the ST segment of fetal ECG may allow us to monitor continuously the metabolic integrity of one of the three major organs preferentially favored by the fetal compensatory responses to asphyxia. The value of such an approach to the prevention of central

nervous system damage because of intrapartum asphyxia needs to be evaluated in prospective clinical studies.

Ramiah Ramasubramanian, MB, BS, Michele Mohajer, MB, BS, Edwin M. Symonds, MD, Mark C. Luxton, MB, ChB, and Alan R. Aitkenhead, MD

Departments of Anesthesia and Obstetrics/Gynecology, University Hospital, Queen's Medical Center, Nottingham NG7 2UH, United Kingdom

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Occupational vibration during pregnancy

To the Editors: Women are entering the workplace in increasing numbers[1] and are diversifying their choice of employment to include jobs in agriculture, construction, forestry, mining, transportation, and heavy industries in which vibration of all or parts of their bodies is significant. As a potential environmental hazard for a subset of working women who are pregnant, occupational vibration has received very little attention.

Although it is to be expected that prolonged exposure to vibration could result in increased fatigue and diminished performance,[2,3] certain conditions already present in pregnancy, such as abdominal and lower back pain, could be aggravated.[4] Low-frequency vibrations easily pass through the abdominal wall, uterine tissues, and fetal fluids[5] and could also have some adverse effects on the developing embryo or fetus.

Jobs in which the whole body is exposed to vibrations of 4 to 8 Hz are most risky, for it is in this range that the abdominal segment "resonates"[3] and is least likely to resist the oscillation forces that are imparted by machines on which women stand and sit or against which they lean.

As a group, obstetricians have done the most to publicize the fact that vibratory mechanical forces that are applied to the abdominal skin of pregnant women are easily transmitted to the fetus; in most cases, this results in fetal movements and cardioacceleration.[6,7] The fetal vibroacoustic stimulation test appears to be useful in confirming the presence of a healthy fetus that is destined for a healthy birth.[8] It is now time to broaden this clinical interest to include the effects of occupational vibration on pregnancy outcome. Unfortunately, the usual indices of outcome—premature birth, birth defects, Apgar and other neonatal scores—may not be sensitive enough to assess this type of exposure accurately.

Until a definite answer can be given to the question, "Does occupational vibration affect the course and outcome of pregnancy?" it is important to lobby for increased worker protection, worker education, and applied research in this emerging and growing area of concern for working pregnant women.

Robert M. Abrams, PhD

University of Florida, Department of Obstetrics and Gynecology, Box J-294 JHMHC, Gainesville, FL 32610-0294

Donald E. Wasserman, MSEE

Wasserman and Associates, Cincinnati, OH 45242

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Group B streptococcal disease

To the Editors: We read the interesting article by Silver et al. (Silver HM, Gibbs RS, Gray BM, Dillon HC. Risk factors for perinatal group B streptococcal disease after amniotic fluid colonization. AM J OBSTET GYNECOL 1990;163:19-25) and we would like to add some data from our experience,[1] which could clear up some points.

In the first place, while studying at-risk patients (intraamniotic infection or rupture membranes >10 hours), the authors found that 81% of group B streptococcal vaginal carriers had group B streptococci in amniotic fluid during labor. They stated, "It is not possible to determine whether this is a usual occurrence or simply related to the method of patient selection." Because the reported value is similar to the 86% we found in our study of amniotic fluids 2 hours after rupture of the membranes in unselected intrapartum group B streptococcal carriers,[1] the majority of whom show no signs of intraamniotic infection, we think we may conclude that it is really a usual occurrence, even in patients without evidence of intraamniotic infection.

Similarly, the neonatal colonization rate reported when group B streptococci were cultured in amniotic fluid was 76%, compared with 69% in our work when they were cultured in amniotic fluid and/or in the vagina during delivery.

Finally, we would like to point out an interesting finding of the authors not mentioned in the discussion: 12% of patients had group B streptococci in amniotic fluid

but not in the vagina, a fact that is also consistent with the 19% reported by us[1] of the interpretation complex.

R. Matorras, MD, PhD

Department of Obstetrics and Gynecology, University of País Vasco, Hospital de Cruces, Baracaldo-Vizcaya, Spain

A. Garcia-Perea, MD, PhD

Department of Microbiology

J.A. Usandizaga, MD, PhD

Department of Obstetrics and Gynecology

F. Omeñaca, MD

Department of Pediatrics, Hospital "La Paz," Autonomous University of Madrid, Madrid, Spain

REFERENCE

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Reply

To the Editors: We would like to thank Drs. Matorras, Garcia-Perea, and Usandizaga for their interest in our article and for bringing their data to our attention. It is very intriguing that the findings in their group of unselected patients was so complementary to the findings in our high-risk group of patients. This underscores the need to further define the mechanism of transformation from asymptomatic colonization to overt infection with group B streptococcus if a successful prophylactic regimen is to be devised.

Helayne M. Silver, MD

Departments of Obstetrics and Gynecology, University of California, Davis, 1621 Alhambra Blvd., Sacramento, CA 95816-7051

Ronald S. Gibbs, MD

Department of Obstetrics and Gynecology, University of Colorado, 4200 E. 9th St., Denver, CO 80262



ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

World Symposium of Perinatal Medicine, October 28-31, 1991, Sheraton Palace Hotel, San Francisco, California. For information contact: Contemporary Forums, 530 La Gonda Way, Suite E, Danville, CA 94526. Tel.: (415) 820-2800.

International Cervical Cancer Symposium, Hotel La Toc, Castries, Saint Lucia, West Indies, November 26-30, 1991. For information contact: Dr. Jean Paul Ryst, Ministry of Health, Chaussée Road, Castries, Saint Lucia, West Indies. Tel.: (809 45) 32 668. Fax: (809 45) 31 080 or (809 45) 25 655.

"AIDS Update for Primary Care: Early Detection for Early Intervention" (includes 3-hour symposium on Ob-Gyn issues), Thursday May 23, 1991 (12 PM to 8 PM), Alta Bates-Herrick Hospital Auditorium, 3001 Colby St., Berkeley, CA 94705. Conference fee: $125 for physicians; $70 for nurses and residents. This conference is approved for 7.5 hours category 1 credit by the CMA; applied for 7.5 cognates to ACOG; 7.5 prescribed hours to the American Academy of Family Practice; California State Psychological Association; Board of Dental Examiners. Sponsored by Alta Bates-Herrick Hospital and East Bay AIDS Center. For information contact: Mary Grim, Medical Education Coordinator, at (415) 540-1420.

New Frontiers in Pelvic Laparoscopic Surgery, Lille, France, August 29-31, 1991. For information contact: Denis Querleu, MD, Pavillon Paul Gellé, 91 Ave. Julien Lagache, 59100 Roubaix, France. Tel.: 20 99 32 54. Fax: 20 99 30 02.

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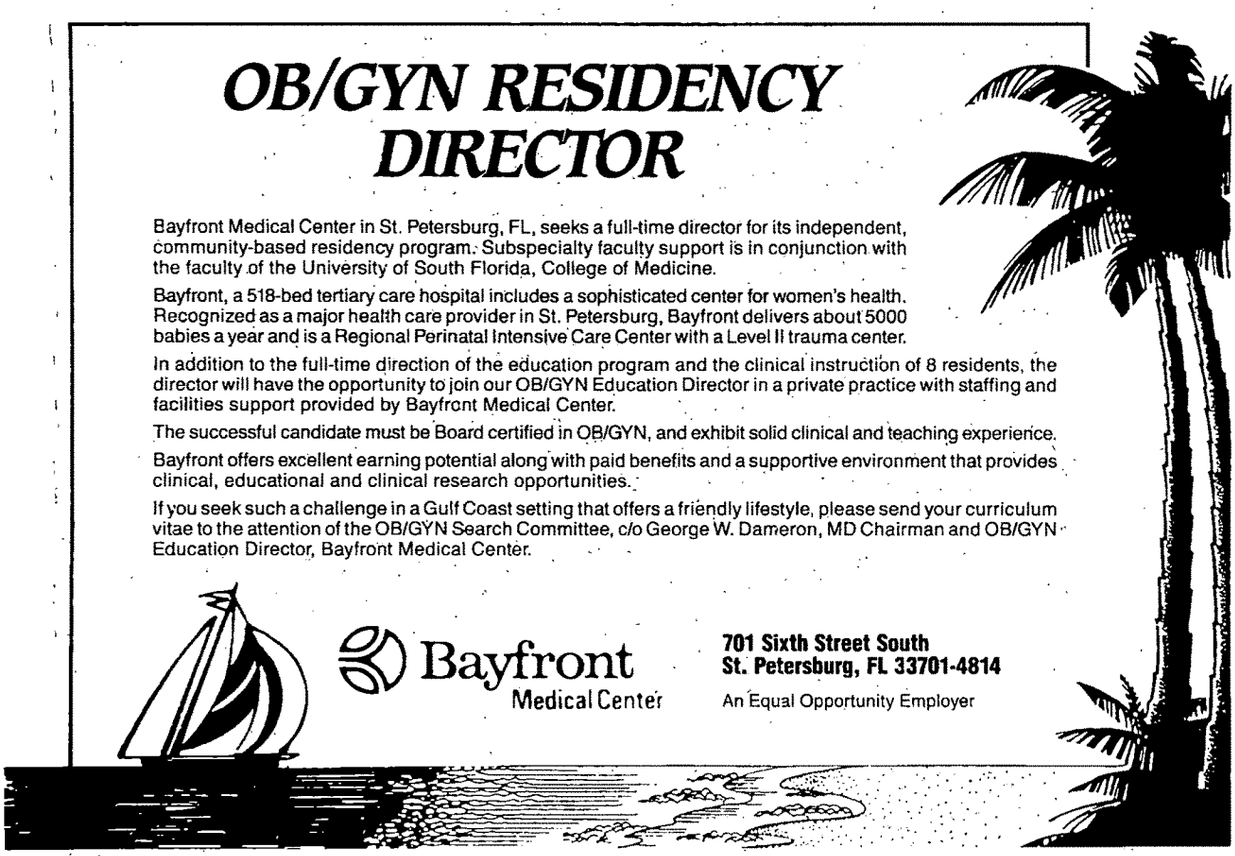
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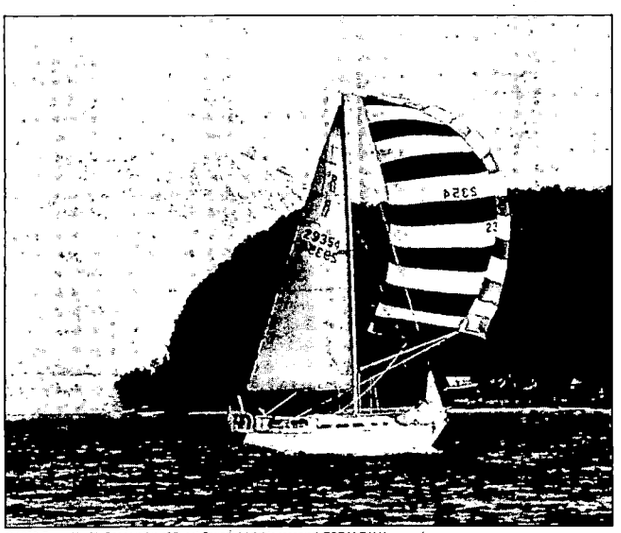
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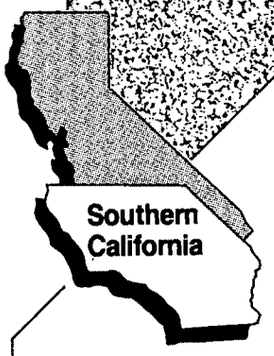
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University of Pittsburgh
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Department of Obstetrics and Gynecology
Assistant Professor of Clinical Obstetrics and Gynecology

Immediate opening for a Board-certified or Board-eligible perinatologist with a special interest in training in public health, Masters Degree preferred. The successful candidate will be prepared to develop epidemiologic research and outreach programs. Salary will be commensurate with training and experience. Compensation includes excellent fringe benefit plan. Interested candidates should submit a curriculum vitae to: Marvin C. Rulin, M.D., Chairman, Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, c/o Magee-Womens Hospital, Forbes and Halket Street, Pittsburgh, PA 15213.

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Maine Medical Center is seeking a Perinatologist to join its busy two person division in Maine's only tertiary perinatal center. The center features a new labor and delivery suite and NICU as well as an established high resolution ultrasound diagnostic unit performing invasive diagnostic procedures.

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Western Massachusetts staff model Health Maintenance Organization is seeking two full-time, Board Certified/Board Eligible Obstetrician/Gynecologists to join our expert staff of five physicians and five midwives.

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Attractive Pioneer Valley location with fine educational, cultural and recreational opportunities. Excellent salary and comprehensive benefit program. Please send curriculum vitae to: Oded Rencus, M.D., Chief of OB/GYN, 305 Bicentennial Highway, Springfield, MA 01118 or call (413) 594-3111.

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Private Practice

Obstetrician Gynecologist

Private practice opportunity for BC/BE Obstetrician/Gynecologist to join or affiliate with a six member Board Certified OB/GYN group in Sioux Falls, South Dakota.

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Sioux Falls, South Dakota 57105
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PENNSYLVANIA HOSPITAL MATERNAL-FETAL MEDICINE FELLOWSHIP

The accredited fellowship in Maternal-Fetal Medicine of the Pennsylvania Hospital is seeking applicants for a position as a first year fellow beginning July 1991. The fellowship is a 2 year clinical and research-oriented program. Clinical services are based at Pennsylvania Hospital, a Level III referral center with a staff of 8 perinatologists who operate a large network of antenatal testing units, including genetics services and invasive ultrasonography. The position includes an academic appointment in Obstetrics and Gynecology at the University of Pennsylvania. Applicants must have completed an approved 4 year residency program in Obstetrics and Gynecology. For more information, please write to:

Stuart Weiner, M.D.
Section Chief,
Maternal-Fetal Medicine
Department of OB/GYN
Pennsylvania Hospital
8th & Spruce Streets
Philadelphia, PA 19107

Deadline for applications is **June 17, 1991.**

Pennsylvania Hospital is an affiliate of The University of Pennsylvania and is an affirmative action/equal opportunity employer.

OB/GYN Position Available

Oklahoma's largest multispecialty group is offering an outstanding opportunity for a sixth BC/BE Obstetrician/Gynecologist.

As a 90-plus member physician-owned group, the Oklahoma City Clinic offers an exceptional two-year guarantee plus incentive, paid malpractice, health, life, disability, retirement, 401K-plan, CME fund and stockholder eligibility after two years with no buy-in.

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REPRODUCTIVE ENDOCRINOLOGIST and DIRECTOR OF OB/GYN RESEARCH

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VICTOR HALITSKY, M.D.
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NASSAU COUNTY MEDICAL CENTER
2201 Hempstead Tpke.
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NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting physicians in a variety of specialties for positions throughout our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

Obstetrician/Gynecologist

BC/BE Obstetrician/Gynecologist sought to join small congenial clinic setting within established pre-paid multispecialty group. Practice consists of general obstetrics and gynecology, high-risk obstetrics and infertility. Expertise in colposcopy, laparoscopy, hysteroscopy and laser are welcomed. Association with resident staff and teaching opportunities available.

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For more information, send CV to: Victor David, M.D., Chief, Obstetrics and Gynecology, The Permanente Medical Group, 901 Nevin Avenue, Richmond, CA 94801. EOE

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Send CV in confidence to:

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The Department of Obstetrics and Gynecology of S.U.N.Y., Health Science Center at Syracuse is offering a one-year fellowship in Pelvic Surgery for qualified candidates.

The Fellowship will include comprehensive training in benign and malignant pelvic surgery, infertility surgery, laser surgery (abdominal, endoscopic and external genitalia), female urology and endoscopy (laparoscopy, hysteroscopy and colposcopy). The program is designed to provide clinical experience and expertise in gynecologic surgery. Research opportunities are available.

Positions are available beginning July 1, 1991.

Qualifications include ABOG eligible/certified status and eligibility for full NY State licensure.

Requests for applications can be sent to:

Michael S. Baggish, M.D.
Professor and Chairman
Department of OB/GYN
S.U.N.Y., Health Science Center
736 Irving Avenue
Suite 308 West Tower
Syracuse, NY 13210

SUNY, Health Science Center is an AA/EEO Employer

A full-time position is available in the Department of Obstetrics and Gynecology, University of California, Davis, Medical Center, Sacramento. Applicants should be Board eligible or certified in Obstetrics and Gynecology. Applicants must possess or be eligible for medical licensure in the State of California. Applicants must demonstrate experience and competence in teaching effectiveness and research related activities or potential for such activities. Special interest in the area of urogynecology, adolescent OB/GYN or sexual dysfunction is desired.

Responsibilities of this position include inpatient and outpatient case management, participation in teaching activities with residents, interns and medical students, development and/or participation in research activities and involvement in University and Hospital committees.

Send curriculum vitae and five references to:

R. Jeffrey Chang, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
1621 Alhambra Boulevard, Suite 2500
Sacramento, California 95816

The final date for receipt of applications is **June 1, 1991.**

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FACULTY POSITION

The University of Illinois College of Medicine at Peoria*

OBSTETRICIAN/GYNECOLOGIST

A full-time faculty position is available for a physician with board certification or eligibility by the American Board of Obstetricians and Gynecologists. Candidates should be interested in clinical care and medical student and resident education. Competitive compensation and excellent benefit package. Applications will be considered until the positions are filled. Send inquiry and curriculum viate to:

Thomas L. Gross, M.D.
Associate Professor and Chairman
Department of Obstetrics and Gynecology
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One Illini Drive, Box 1649
Peoria, Illinois 61656

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FELLOWSHIP
MATERNAL-FETAL MEDICINE
JULY, 1992

The Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University MacDonald Womens Hospital, University Hospitals of Cleveland, and Case Western Reserve University, at the instructor level beginning July 1, 1992. Applicants should desire a career in academic medicine and must be Board certified/eligible by the American Board of Obstetrics and Gynecology.

Fellows will be involved in teaching medical students and house officers. The fellowship program emphasizes clinical and basic research in clinical perinatology with a particular interest in ultrasound, and perinatal diagnosis. The individual will also be responsible for some general duties in obstetrics and gynecology.

Applicants should send a curriculum vitae to:

Method Duchon, M.D.
Acting Director, Division of Maternal-Fetal Medicine
University MacDonald Womens Hospital
University Hospitals of Cleveland
2074 Abington Road
Cleveland, Ohio 44106
(216) 844-3304

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NORTHERN CALIFORNIA

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REPRODUCTIVE ENDOCRINOLOGIST

• SAN JOSE •

We are currently seeking a Reproductive Endocrinologist to establish and run a reproductive endocrinology subdivision in the department. Duties would include the full spectrum of reproductive endocrinology, outpatient and surgical management of infertility. Fellowship certification would be preferred. The department currently has 17 physicians and is still growing. We offer a generous benefits package including full coverage malpractice insurance. San Jose has beautiful weather and excellent access to Bay Area recreational and cultural activities.

For more information, please contact: Jerome L. Nehemiah, M.D., Chief, Department of OB/GYN, The Permanente Medical Group, Inc., 260 International Circle, Dept. AJOBG, San Jose, CA 95119, or call collect (408) 972-6180. EOE



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FACULTY OPENINGS IN OBSTETRICS & GYNECOLOGY FOR GENERALISTS/PERINATOLOGISTS

Full time faculty positions at the University of South Alabama, Department of Obstetrics & Gynecology. Excellent opportunity for teaching, research and clinical service. Individuals must be board eligible or certified in Specialty/Sub-specialty. The academic rank and salary will be dependent upon the training and experience of the applicant. These positions are available immediately.

Please contact:

Ian H. Thorneycroft, Ph.D., M.D.
Professor and Chairman
Department of Ob-Gyn
University of South Alabama
College of Medicine
2451 Fillingim Street
Mobile, Alabama 36617
(205) 470-5815

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U.T. Southwestern Medical Center at Dallas

Molecular neuroendocrinology/ Molecular neuroscience

Full time faculty position available for applicant with Ph.D. in biochemistry. Five years experience in molecular biology. Proven publication record in: 1) catecholamien synthesis, 2) neuronal cultures, 3) gene regulation and related techniques (particularly nuclease digestion of DNA, RNA transcript, nuclease protection assay of mRNA), 4) protein synthesis, 5) analysis of enzymes (particularly enzymatic assays, kinetic analysis, quantification using immunoblotting). Undergraduate teaching responsibilities.

Salary and academic appointment commensurate with experience. Interested applicants should send cover letter, curriculum vitae, and summary of related research to: John C. Porter, Ph.D., Professor of Obstetrics and Gynecology and Physiology, Department of Obstetrics and Gynecology, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235-9032. An Equal Opportunity/Affirmative Action Employer.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Obstetrics and Gynecology
Assistant Professor Level (Tenure Track)

The University of California, San Francisco, Department of Obstetrics, Gynecology and Reproductive Sciences, is seeking a full-time obstetrician-gynecologist. May have clinical expertise in either gynecology or perinatology and should be well-trained in contemporary investigative reproductive biology, ideally in immunology, microbiology or virology.

Applicant must demonstrate aptitude for, and interest in, teaching and research, and be capable of mounting his or her own research program.

Please send curriculum vitae to:

Daniel V. Landers, M.D.
Department of Obstetrics, Gynecology and Reproductive Sciences
San Francisco General Hospital, Room 6D14
1001 Potrero Avenue
San Francisco, CA 94110

Obstetrics, Gynecology and Reproductive Sciences
Assistant Professor Level (Tenure Track)

The University of California, San Francisco, Department of Obstetrics, Gynecology and Reproductive Sciences is seeking a full-time obstetrician/gynecologist with training and expertise in molecular virology (papillomavirus, oncogenes).

Applicant should be capable of mounting an independent research program and demonstrate an aptitude and interest in teaching and clinical care.

Please send curriculum vitae to:

Russell K. Laros, Jr., M.D.
Department of Obstetrics, Gynecology and Reproductive Sciences
University of California, San Francisco
San Francisco, California 94143-0132

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CHIEF OF PERINATOLOGY

West Virginia University School of Medicine seeks a board certified perinatologist to establish and direct a program of regional perinatal care. Successful candidate will hold faculty rank at West Virginia University based on qualifications. The Medical School and Department of Obstetrics and Gynecology are committed to supporting the development of a program with innovative approaches to the improvement of regional perinatal health in West Virginia. The position includes support for (recruitment of) additional personnel and research. The deadline date for receipt of applications is July 31, 1991.

Send C.V. to:

Mark Gibson, M.D.
c/o Marlene Hershman
Office of the Chairman
Department of Obstetrics and Gynecology
West Virginia School of Medicine
Health Sciences Center
Morgantown, West Virginia 26506

West Virginia University is an Equal Opportunity Employer.

GYN ONCOLOGIST

New Hanover Regional Medical Center, located in beautiful, historic, oceanside Wilmington, North Carolina is seeking a board certified or active candidate Gyn oncologist, preferably with academic experience to assist four other full-time faculty in supervising the training of residents and students. New Hanover Regional Medical Center is a 500 bed major referral hospital for Southeastern North Carolina with Ob/Gyn and other graduate medical education programs affiliated with the University of North Carolina School of Medicine. The Ob/Gyn program is approved for twelve residents and full-time faculty are supported by faculty visits from UNC-Chapel Hill as well as an enthusiastic private attending staff. Teaching responsibilities also include medical students from the University of North Carolina at Chapel Hill. Successful candidate will be eligible for tenure track appointment at UNC School of Medicine. Academic appointment and salary dependent on training and experience. Please submit curriculum vitae and references to:

Chairman, Search Committee in Ob/Gyn
Area Health Education Center
New Hanover Regional Medical Center
2131 South 17th Street
Wilmington, North Carolina 28402

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OBSTETRICS & GYNECOLOGY FACULTY POSITIONS
The University of Texas Southwestern Medical Center at Dallas

GENERALISTS IN OBSTETRICS & GYNECOLOGY

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MATERNAL-FETAL MEDICINE/REPRODUCTIVE ENDOCRINOLOGY

Full time positions available to qualified applicants who have completed an accredited residency and a 2-year Fellowship in either Maternal Fetal Medicine or Reproductive Endocrinology and are eligible for Texas licensure. Responsibilities will include patient care; graduate and undergraduate teaching; research opportunities and participation in departmental continuing medical education efforts.

GYNECOLOGIC ONCOLOGY

Full time positions available to qualified applicants who have completed an accredited residency and a 2-year Fellowship in Gynecologic Oncology and are eligible for Texas licensure. Responsibilities will include patient care including surgery and chemotherapy of gynneoplasms; graduate and undergraduate teaching; research opportunities and participation in departmental continuing medical education efforts.

Salary and academic rank commensurate with curriculum vitae and experience.

Interested persons send curriculum vitae to: F. Gary Cunningham, M.D., Professor and Chairman, Department Ob-Gyn, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235-9032.

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Fellowship In Reproductive Genetics

The Division of Reproductive Genetics, Department of Obstetrics and Gynecology at The University of Tennessee, Memphis, is offering a two- or three-year fellowship in reproductive genetics beginning July 1991. The fellowship program is approved by the American Board of Medical Genetics. Applicants must have completed a residency in obstetrics and gynecology, and eligibility for medical licensure in Tennessee is required. The fellowship is designed to provide a comprehensive experience in clinical genetics (counseling, prenatal diagnosis, amniocentesis, chorionic villus sampling, fetal blood sampling, ultrasonography, dysmorphology, teratology, alpha-fetoprotein screening). The program emphasizes laboratory training and research in cytogenetics, DNA analyses, inborn errors of metabolism, immunogenetics and population genetics. Fellows are actively involved in clinical and laboratory research projects. Applicants should send a curriculum vitae to: UT Medical Group, Employment Office, 66 N. Pauline, Memphis, TN 38105.

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UTMB **University of Texas Medical Branch Galveston, Texas**

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Obstetrician-Gynecologist

- Board eligibility or certification in obstetrics and gynecology required.
- Academic rank and salary commensurate with experience and qualifications.

Curriculum Vitae should be sent to:

Garland D. Anderson, M.D.
Jennie Sealy Smith Professor & Chairman
Department of Obstetrics & Gynecology
The University of Texas Medical Branch
Galveston, Texas 77550-2776

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CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

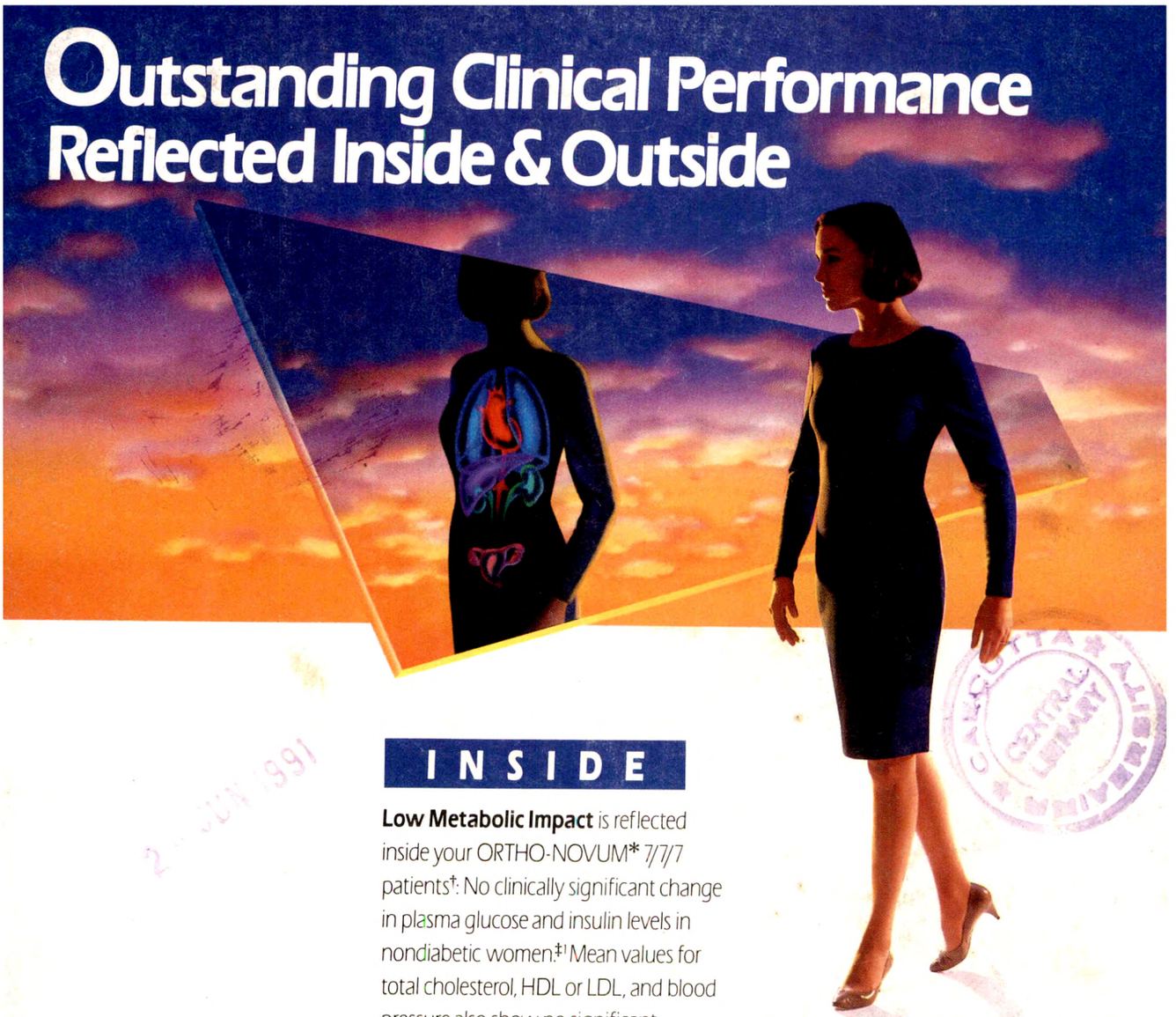
The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Revised 9/89

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

ORTHO

Outstanding Clinical Performance Reflected Inside & Outside



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INSIDE

Low Metabolic Impact is reflected inside your ORTHO-NOVUM* 7/7/7 patients†: No clinically significant change in plasma glucose and insulin levels in nondiabetic women.‡[1] Mean values for total cholesterol, HDL or LDL, and blood pressure also show no significant change.§

Excellent Cycle Control is another reflection of outstanding performance with ORTHO-NOVUM 7/7/7. You can expect low discontinuation rates due to breakthrough bleeding, spotting, and amenorrhea.

OUTSIDE

Low Incidence of Androgenic Side Effects—such as acne, oily skin, or hirsutism—is a visible reflection that your patients may notice; another is no clinically significant change in mean body weight. Inside and outside, the outstanding performance of ORTHO-NOVUM 7/7/7 is also an excellent reflection on you.

*Trademark

†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

‡Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents.

§An increase in blood pressure has been reported in women taking oral contraceptives.

1. Spellacy WN, Ellingson AB, Tsibris JCM. Glucose and insulin levels after six months of treatment with a triphasic oral contraceptive containing ethinyl estradiol and norethindrone. *J Reprod Med.* 1989;34(8):540-542.

Please see complete Prescribing Information, a brief summary of which is on the preceding page.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

ORTHO

Low incidence of androgenic side effects

21- and 28-day regimens

ORTHO-NOVUM 7/7/7 Tablets

TRADEMARK

norethindrone/ethinyl estradiol

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(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL®

Uniquely phased for all phases of a woman's reproductive life

ORAL CONTRACEPTIVE AGENTS: BRIEF SUMMARY

DESCRIPTION

TRI-NORINYL 21-DAY Tablets provide an oral contraceptive regimen of 7 blue tablets followed by 9 yellow-green tablets and 5 more blue tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg and each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg.

TRI-NORINYL 28-DAY Tablets provide a continuous oral contraceptive regimen of 7 blue tablets, 9 yellow-green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg, each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert ingredients.

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions: Thrombophlebitis or thromboembolic disorders • A past history of deep vein thrombophlebitis or thromboembolic disorders • Cerebral vascular or coronary artery disease • Known or suspected carcinoma of the breast • Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia • Undiagnosed abnormal genital bleeding • Cholestatic jaundice of pregnancy or jaundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors • Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholesterolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estrogen. The effects of long-term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the *ratio* of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide not only a measure of the relative risk but a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

a. Myocardial infarction

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS,** section 9). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2- to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970's—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral contraceptive use to women who do not have the various risk factors listed in this labeling.

Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular disease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users.

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0.075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed earlier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The incidence of risk also was reported to increase with continued use and among older women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens.

Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. LIPID DISORDERS

Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention.

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected by oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measurerd by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.

c. Other binding proteins may be elevated in serum.

d. Sex steroid binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged.

e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives.

9. CARCINOGENESIS

See **WARNINGS** section.

10. PREGNANCY

Pregnancy Category X. See **CONTRAINDICATIONS** and **WARNINGS** sections.

11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

INFORMATION FOR THE PATIENT

See **PATIENT LABELING.**

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **WARNINGS** section): Thrombophlebitis • Arterial thromboembolism • Pulmonary embolism • Myocardial infarction • Cerebral hemorrhage • Cerebral thrombosis • Hypertension • Gallbladder disease • Hepatic adenomas, carcinomas or benign liver tumors

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis • Retinal thrombosis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea • Vomiting • Gastrointestinal symptoms (such as abdominal cramps and bloating) • Breakthrough bleeding • Spotting • Change in menstrual flow • Amenorrhea • Temporary infertility after discontinuation of treatment • Edema • Melasma which may persist • Breast changes: tenderness, enlargement, secretion • Change in weight (increase or decrease) • Change in cervical erosion and secretion • Diminution in lactation when given immediately postpartum • Cholestatic jaundice • Migraine • Rash (allergic) • Mental depression • Reduced tolerance to carbohydrates • Vaginal candidiasis • Change in corneal curvature (steepening) • Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome • Cataracts • Changes in appetite • Cystitis-like syndrome • Headache • Nervousness • Dizziness • Hirsutism • Loss of scalp hair • Erythema multiforme • Erythema nodosum • Hemorrhagic eruption • Vaginitis • Porphyria • Impaired renal function • Hemolytic uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral contraceptive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: • Increased menstrual cycle regularity • Decreased blood loss and decreased incidence of iron deficiency anemia • Decreased incidence of dysmenorrhea

Effects related to inhibition of ovulation: • Decreased incidence of functional ovarian cysts • Decreased incidence of ectopic pregnancies

Effects from long-term use: • Decreased incidence of fibroadenomas and fibrocystic disease of the breast • Decreased incidence of acute pelvic inflammatory disease • Decreased incidence of endometrial cancer • Decreased incidence of ovarian cancer

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814-H2-170-90

Rev. May 1990

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

Nonsmoking, healthy woman in late-30s wishes to restart relationship with oral contraceptive. Low metabolic and hormonal impact preferred.

Active young female in late teens looking for lasting relationship with OC. Concerned about side effects of acne and bloating.

Career - oriented woman in mid-20s wants to find right OC. Wishes to avoid side effects such as weight gain, headache, and depression. Open to raising a family — but later.

A personalized approach to oral contraceptives.

Because Tri-Norinyl is uniquely phased for all phases of a woman's reproductive life, it's ideal for a wide range of patient types:

Older, nonsmoking patients restarting contraception: The only multiphasic with norethindrone that *lowers* progestin in late cycle to minimize overall hormonal and metabolic impact.

Career-oriented patients delaying childbearing: Reduced progestin helps minimize side effects such as weight gain, headache, and depression.

Younger patients just starting contraception: Low-impact progestin lowers risk of acne and bloating; mid-cycle increase enhances support when breakthrough bleeding is most likely to occur.

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(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL®

Uniquely phased for all phases of a woman's reproductive life

Committed to the OB/GYN and women's healthcare

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see next page for brief summary of prescribing information. ©1990 Syntex Laboratories, Inc. 814-H2-201-90

GORE-TEX® SOFT TISSUE PATCH

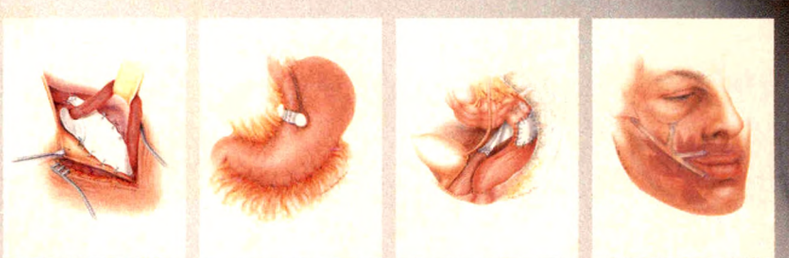
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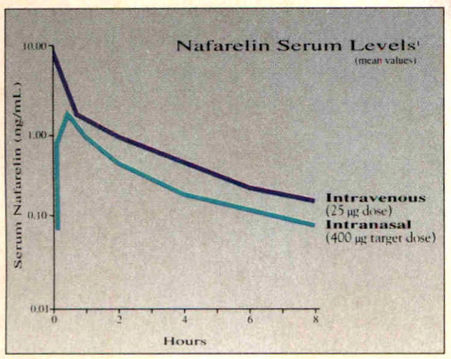
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- Intranasal administration produces nafarelin serum levels comparable to I.V. dosing: In pharmacokinetic studies, SYNAREL was rapidly absorbed into systemic circulation following intranasal administration, with maximum serum concentrations achieved between 10 and 40 minutes.[1]



- B.I.D. controlled-dose intranasal administration suppresses serum estradiol to levels that result in outstanding clinical efficacy, while producing minimal impact on bone and a predictable hypoestrogenic profile.†
- Facilitates ease of patient management should allergic or other adverse events occur.
- Retreatment cannot be recommended since safety data for retreatment are not currently available.

1. Syntex document #829-222-A.

*Gonadotropin-releasing hormone analog.

†There is a small, largely reversible loss in bone density with 6-month treatment. Use of SYNAREL for longer than the recommended 6 months or in the presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Pregnancy should be ruled out before therapy with SYNAREL is initiated, and patients should be advised to use a non-hormonal method of contraception when taking SYNAREL.

Please see adjacent page for brief summary of prescribing information.

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SYNAREL®

(NAFARELIN ACETATE)

NASAL SOLUTION, 2 MG/ML

Setting The Pace In Endometriosis Therapy

BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloride, glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.

INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.

CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.

WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.

When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.

PRECAUTIONS Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. Patients missing successive doses may experience breakthrough bleeding.

2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients.

3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician.

4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients.

5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content.

6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption.

7. Retreatment cannot be recommended since safety data beyond 6 months are not available.

Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.

Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.

Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.

Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (±SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

SYNTEX

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PALO ALTO, CA 94304

February 1990
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SYNAREL®
(NAFARELIN ACETATE)
NASAL SOLUTION, 2 MG/ML

CURRENT PROBLEMS IN OBSTETRICS, GYNECOLOGY AND FERTILITY

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May
Part 1
1991

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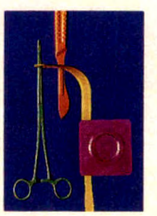
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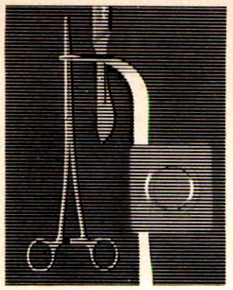
(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

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Count on

CEFOTAN® IM/IV

(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary tract infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).
Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli.*
Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli.*
Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).
Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).
Bone and joint infections caused by *Staphylococcus aureus.**
*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.

PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be

developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).
Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).
Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).
Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).
Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:} \quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.

1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

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The N-terminal and C-terminal portions of the atrial natriuretic factor prohormone increase during preeclampsia 1197
Rhene W. Merkouris, MD, Frank C. Miller, MD, Val Catanzarite, MD, PhD, J. Gerald Quirk, Jr., MD, PhD, Lee A. Rigg, MD, PhD, and David L. Vesely, MD, PhD
Little Rock, Arkansas, and Tampa, Florida

Both the amino terminus (N-terminus) and the carboxy terminus (C-terminus) of the atrial natriuretic factor prohormone markedly increase during preeclampsia; both decrease immediately post partum.

Gestational age in relation to marine *n*-3 fatty acids in maternal erythrocytes: A study of women in the Faroe Islands and Denmark 1203
Sjúrður F. Olsen, MD, MSc, Harald S. Hansen, Dr.Scient., Steffen Sommer, MD, Benny Jensen, Lic.Tecn., Thorkild I.A. Sørensen, Dr.Med.Sci., Niels J. Secher, MD, and Petur Zachariassen, Cand.Scient.
Tórshavn, Faroe Islands, and Aarhus and Copenhagen, Denmark

The level of marine *n*-3 fatty acids in erythrocytes, which reflects dietary intake, is associated with gestational age in Danish women but not in Faroese women.

Recurrent failure of in vitro fertilization: Role of the hemizona assay in the sequential diagnosis of specific sperm-oocyte defects 1210
Sergio Oehninger, MD, Anibal A. Acosta, MD, Lucinda L. Veeck, MLT, Robert Brzyski, MD, PhD, Thinus F. Kruger, MD, Suheil J. Muasher, MD, and Gary D. Hodgen, PhD
Norfolk, Virginia, and Tygerberg, Republic of South Africa

The hemizona assay in sequence with other bioassays and in vitro fertilization results can provide specific sperm defect analysis in cases of fertilization failure.

Umbilical vein administration of oxytocin for the management of retained placenta: Is it effective? 1216
Maarten G.P. Huber, MD, Hajo I.J. Wildschut, MD, PhD, Kees Boer, MD, PhD, Gunilla Kleiverda, MD, and Frans J. Hoek, PhD
Amsterdam, The Netherlands, and Bristol, United Kingdom

In a multicenter randomized controlled trial involving 220 women with retained placenta, intraumbilical injection of oxytocin did not reduce either the percentage of placentas manually removed or the blood loss in the third stage.

The role of the transcerebellar view in the detection of fetal central nervous system anomaly 1220
Lyndon M. Hill, MD, Jerry G. Martin, MD, Joanne Fries, RDMS, and Joyce Hixson, RDMS
Pittsburgh, Pennsylvania

A transcerebellar view should be incorporated into all obstetric ultrasonographic examinations.

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To be continued...



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CA 125 levels in abruptio placentae 1225
Barry R. Witt, MD, Rachel Miles, MD, Gordon C. Wolf, PhD, MD, George T. Koulianos, MD, and Ian H. Thorneycroft, PhD, MD
New Orleans, Louisiana

Patients with bleeding in late pregnancy because of abruptio placentae had significantly elevated serum CA 125 levels compared with similar subjects with vaginal bleeding of other causes.

Management of severe preeclampsia detected in early labor by prostaglandin A_1 or dihydralazine infusions 1229
Mokhtar K. Toppozada, MD, Emad Darwish, MD, and Azza A.T. Barakat, MS
Alexandria, Egypt

Both prostaglandin A_1 and dihydralazine infusions induce a similar hypotensive response in severe preeclampsia encountered in early labor; the more gradual reduction of blood pressure and shorter time to delivery with prostaglandin A_1 are additional clinical advantages.

The intrauterine probe electrode 1233
Thomas H. Strong, Jr., MD, Richard H. Paul, MD, George D. Park, Yi Cheng, PhD, Donald E. Lewis, Dorothy F. McCart, RN, and Evelyn A. Mueller, RN
Los Angeles and Pomona, California

A device capable of detecting the fetal electrocardiogram for fetal heart rate derivation without fetal skin penetration is described.

Management of radionecrosis of the vulva and distal vagina 1235
William S. Roberts, MD, Mitchel S. Hoffman, MD, James P. LaPolla, MD, Ernesto Ruas, MD, James V. Fiorica, MD, and Denis Cavanagh, MD
Tampa, Florida

Radionecrosis of the vulva requires wide excision and appropriate tissue coverage to accomplish healing.

Cocaine and the use of alcohol and other drugs during pregnancy 1239
Ann P. Streissguth, PhD, Therese M. Grant, MEd, Helen M. Barr, MS, Zane A. Brown, MD, Joan C. Martin, PhD, Dennis E. Mayock, MD, Sharon Landesman Ramey, PhD, and Leejon Moore, MD
Seattle, Washington, and Birmingham, Alabama

Cocaine use during pregnancy was reported by 15% of a teaching hospital sample and 3% of a private hospital sample of mothers at delivery.

Fetal thyroid-stimulating hormone response to maternal administration of thyrotropin-releasing hormone 1244
J.G. Thorpe-Beeston, MD, K.H. Nicolaides, MD, R.J.M. Snijders, MSc, J. Butler, MA, and A.M. McGregor, MD
London, England

Injection of thyrotropin-releasing hormone to pregnant women at 25 to 37 weeks' gestation was associated with a rapid increase in fetal blood thyroid-stimulating hormone concentration.

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In utero diagnosis and management of fetal subdural hematoma 1246
Siegfried Rotmensch, MD, Peter A. Grannum, MD, José A. Nores, MD, Cynthia Hall, MD, Marc S. Keller, MD, Shirley McCarthy, MD, and John C. Hobbins, MD
New Haven, Connecticut

A case of fetal subdural hematoma is presented, and criteria for the in utero diagnosis and guidelines for management are discussed.

Calcium-regulating hormones and osteocalcin levels during pregnancy: A longitudinal study 1248
Katsuyoshi Seki, MD, Noriko Makimura, DMSc, Chieko Mitsui, BS, Junko Hirata, BS, and Ichiro Nagata, MD
Tokorozawa City, Saitama, Japan

Serum parathyroid hormone and osteocalcin values, which were low in early pregnancy, declined toward the middle of pregnancy but increased in late pregnancy.

The relationship between autoantibodies and intrauterine growth retardation in hypertensive disorders of pregnancy 1253
Albert El-Roeiy, MD, Stephen A. Myers, DO, and Norbert Gleicher, MD
Chicago, Illinois

Autoantibodies, especially antiphospholipid antibodies, were associated with intrauterine growth retardation in patients with preeclampsia and chronic hypertension.

Umbilical arterial and venous acid-base and blood gas values and the effect of chorioamnionitis on those values in a cohort of preterm infants 1261
Gary D.V. Hankins, Lt Col, USAF, MC, Russell R. Snyder, Lt Col, USAF, MC, and Edward R. Yeomans, Maj, USAF, MC
San Antonio, Texas

Chorioamnionitis has no significant effect on umbilical arterial acid-base or blood gas values in preterm infants whose courses are otherwise uncomplicated; additionally, chorioamnionitis did not increase the percentage of infants who were born with acidemia.

Cervical human papillomavirus deoxyribonucleic acid and cytologic evaluations in gynecologic outpatients 1265
Pekka Nieminen, MD, Valeria R.X. Soares, MSc, Markku Aho, MD, Ervo Vesterinen, MD, Eeva Savia, MD, Antti Vaheri, MC, and Jorma Paavonen, MD
Helsinki, Finland

Of 2668 gynecologic outpatients, 10.2% tested positive for cervical human papillomavirus deoxyribonucleic acid; 73% of women with positive human papillomavirus deoxyribonucleic acid results had normal cervical cytologic evaluations.

A prospective genetic study of complete and partial hydatidiform moles 1270
Sylvia D. Lawler, MD, Rosemary A. Fisher, PhD, and Joan Dent, SRN
London, England

This prospective genetic study of partial and complete hydatidiform moles relates diagnosis to the risk of development of a gestational trophoblastic tumor.

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The effect of sterile water blocks on low back labor pain 1277
Birgitta Trolle, MD, Margrethe Møller, MD, Hanne Kronborg, and Søren Thomsen, MD
Aalborg, Denmark

In a double-blind randomized study, intracutaneous sterile water blocks significantly diminished low back pain.

Electronic fetal heart monitoring, auscultation, and neonatal outcome 1281
Patricia H. Ellison, MD, Mark Foster, MA, Margaret Sheridan-Pereira, MD, and Dermot MacDonald, MD
Denver, Colorado, and Dublin, Ireland

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Mary D. Nettleman, MD, and Thomas A. Bell, MD, MPH
Iowa City, Iowa, and Seattle, Washington

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Chia C. Pao, PhD, Shu-Min Kao, BS, Han-Chow Wang, MD, and Cagge C. Lee, MD
Taipei, Taiwan, Republic of China

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Birth weights of infants of black and white mothers without pregnancy complications 1299
Thomas C. Hulsey, MSPH, ScD, Abner H. Levkoff, MD, and Greg R. Alexander, MPH, ScD
Charleston, South Carolina, and Minneapolis, Minnesota

Controlling for differences in demographic characteristics and eliminating the potential influence of medical complications of pregnancy, the mean birth weight for black infants was significantly less than that for white infants.

Incidence of genital herpes simplex virus at the time of delivery in women with known risk factors 1303
Patrick M. Catalano, MD, Alice O. Merritt, RN, and Philip B. Mead, MD
Burlington, Vermont

Of pregnant women without symptoms who are at risk for development of maternal genital herpes simplex virus, 2.4% had positive cultures at the time of delivery.

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Honor M. Wolfe, MD, Ivan E. Zador, PhD, Thomas L. Gross, MD, Susan S. Martier, MSSA, and Robert J. Sokol, MD
Detroit, Michigan, and Peoria, Illinois

Maternal body mass index offers little advantage over maternal weight for initial clinical perinatal risk assessment.

Complexes of activated protein C with α_1-antitrypsin in normal pregnancy and in severe preeclampsia 1310
Francisco España, PhD, Juan Gilabert, MD, PhD, Justo Aznar, MD, PhD, Amparo Estellés, MD, PhD, Takao Kobayashi, MD, and John H. Griffin, PhD
Valencia, Spain, and La Jolla, California

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Progress in pathogenesis and management of clinical intraamniotic infection 1317
Ronald S. Gibbs, MD, and Patrick Duff, MD
Denver, Colorado, and Gainesville, Florida

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Randall C. Dunn, MD, Alex J. Steinleitner, MD, and Hovey Lambert, PhD
Houston, Texas, and Miami Beach, Florida

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Endothelin and neuropeptide Y are vasoconstrictors in human uterine blood vessels 1330
Gabriel Fried, MD, PhD, and Ulf Samuelson, MD, PhD
Stockholm, Sweden

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Michael J. Parkes, DPhil, Peter J. Moore, PhD, David R. Moore, PhD, Nicholas M. Fisk, MB, BS, and Mark A. Hanson, DPhil
Reading, England

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Sharon H. Nelson, PhD, and Maya S. Suresh, MD
Galveston, Texas

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Aliza Kalo-Klein, PhD, and Steven S. Witkin, PhD
New York, New York

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Antonella Barbati, PhD, Lorena Broccucci, PhD, Maurizio M. Anceschi, MD, PhD, and Ermelando V. Cosmi, MD
Perugia, Italy

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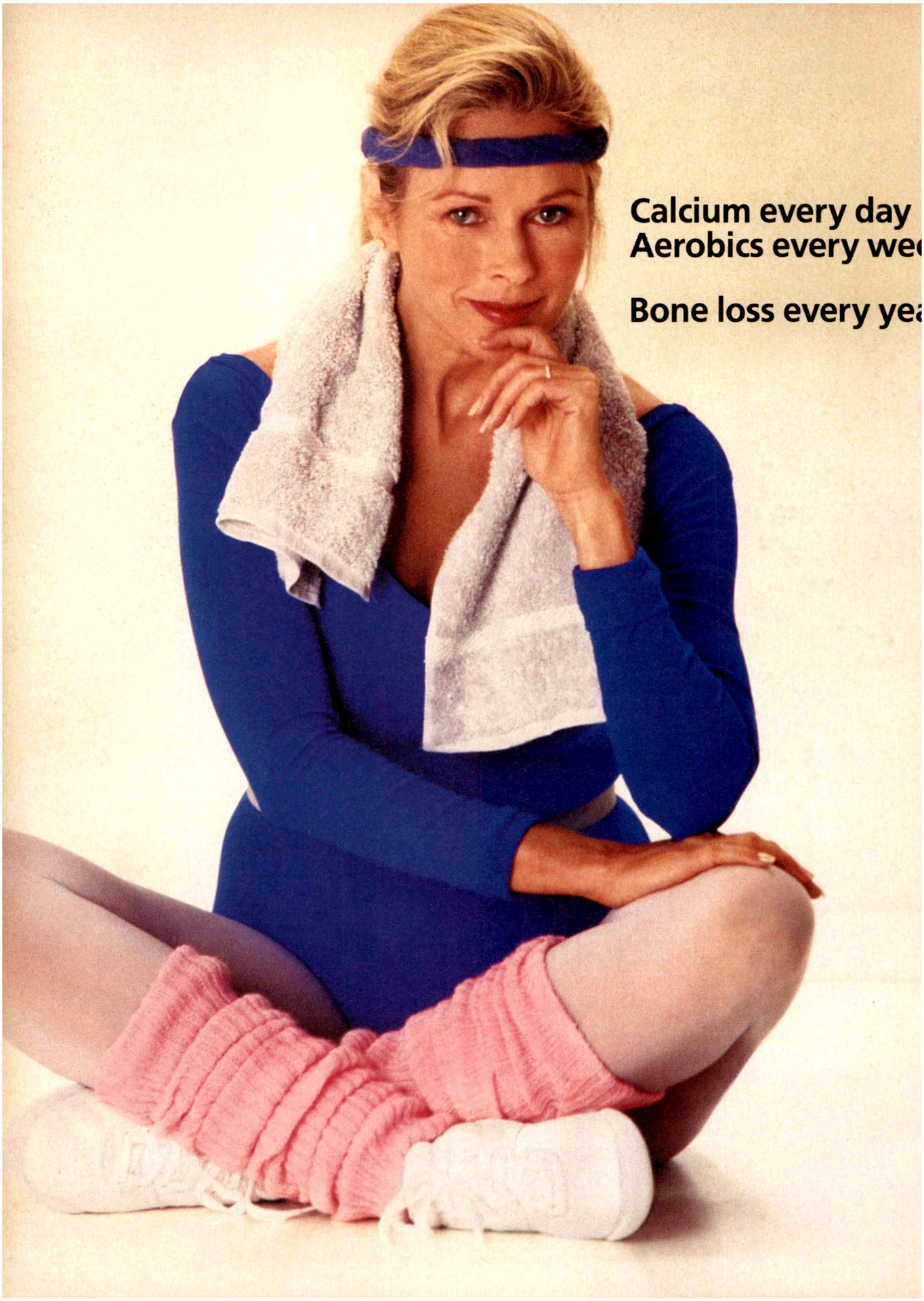
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There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risk, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one yea without another physical examination being performed. Conditions influenced by fluid retention, such as asthma epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develo manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding an mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should b used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associate with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progesti combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, a measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and syntheti estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and live

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATION** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be don only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy; changes in vaginal bleedin pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterin fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breast nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist whe drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hai hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, menta depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyri edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophi vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dos that will control symptoms should be chosen and medication should be discontinued as promptly as possible Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or tape medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.

Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 day followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule i repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downwar according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effectiv control.

Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued a promptly as possible.

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should b monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event persistent or recurring abnormal vaginal bleeding.

Revised August 21, 198

References:

1. Nilas L, Christiansen C, Rødbro P: Calcium supplementation and postmenopausal bone loss. *Br Med J* 198 289:1103-1106. **2.** Riggs BL: Research Directions in Osteoporosis. Scientific Workshop. National Institutes Health. February 9-11, 1987. **3.** Kiel DP, Felson DT, Anderson JJ, et al: Hip fracture and the use of estrogens postmenopausal women. *N Engl J Med* 1987;317:1169-1174. **4.** Christiansen C, Christensen MS, Transbøl Bone mass in postmenopausal women after withdrawal of oestrogen/gestagen replacement therapy. *Lanc* 1981;1:459-461.

Worldwide Leadership in Female Healthcare

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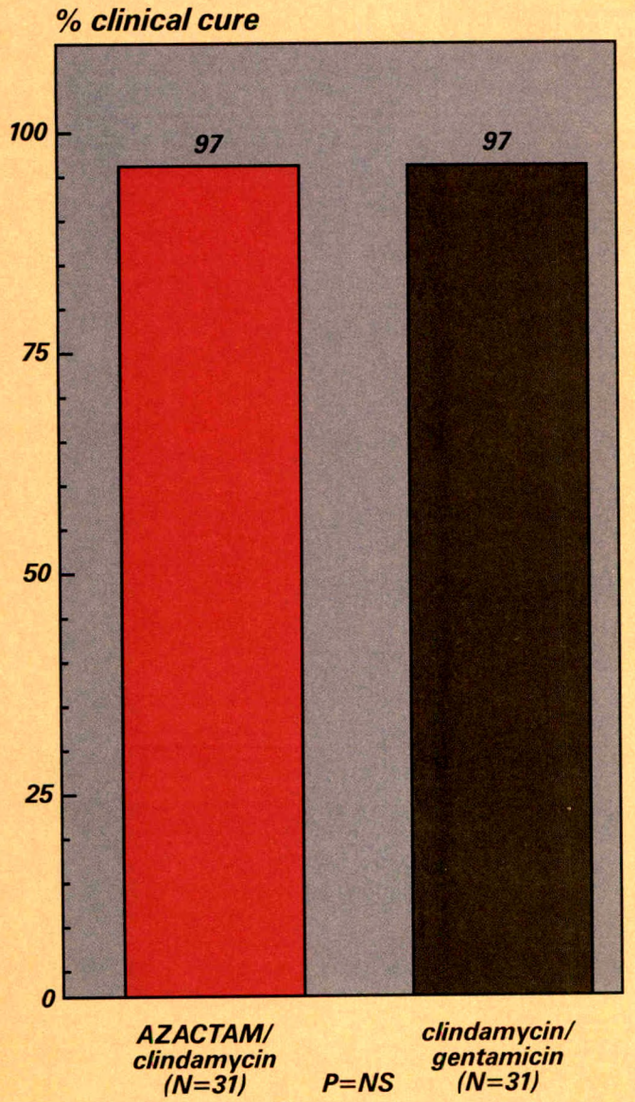
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IN POSTPARTUM ENDOMETRITIS*



A randomized, comparative trial of AZACTAM versus gentamicin, each with clindamycin, for the treatment of endometritis after delivery.[1]

No universal criteria exist that may satisfy a single definition of "cure." Use of this term may vary between clinical settings and investigators, and the reader is urged to consult the reference listed.

Azactam®
aztreonam
delivers...

*Due to susceptible organisms.

†Although a useful guide, in vitro data do not necessarily correlate with therapeutic outcome.

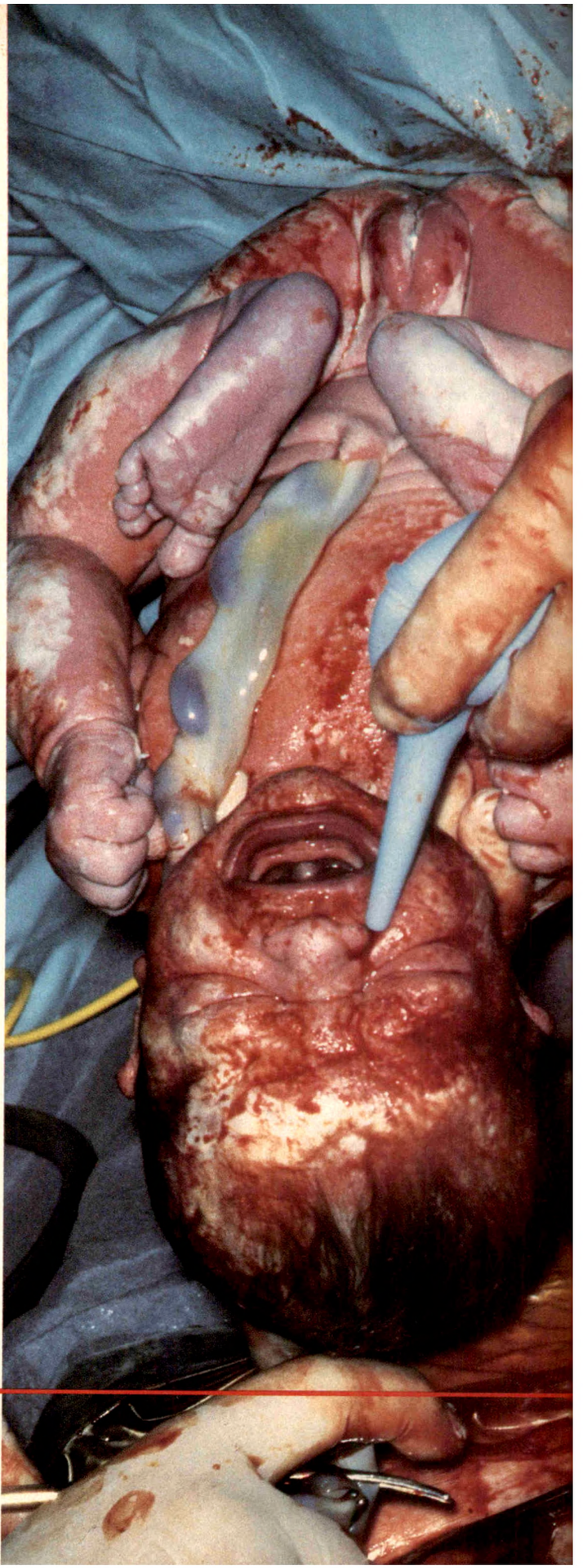
‡Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. See full prescribing information for recommendations on patients with immediate hypersensitivity reactions to penicillins.

Following IV administration, the most commonly seen adverse reactions occurred in less than 2% of patients, including local reactions, diarrhea, nausea, vomiting, and rash.

more than you're expecting...

- *Broad aerobic gram-negative in vitro coverage, including Escherichia coli, Proteus mirabilis, and Enterobacter cloacae.†*
- *Greater than 90% cure rates in combination with clindamycin.[1-3]*
- *No time-consuming, costly, routine therapeutic drug monitoring.*
- *May be admixed in the same bag with clindamycin.*
- *May be used in penicillin-allergic patients.‡*
- *Pregnancy Category B.*

There are no adequate and well-controlled studies in pregnant women. AZACTAM should be used in pregnant women only if clearly needed.



Azactam®
aztreonam

A logical alternative to aminoglycosides.

Please see adjacent page for brief summary of prescribing information.

Azactam®
aztreonam

AZACTAM® FOR INJECTION
Aztreonam For Injection

DESCRIPTION–AZACTAM (aztreonam, Squibb) is the first member of a new class of antibiotics classified as monobactams. AZACTAM is a totally synthetic bactericidal antibiotic with activity against a wide spectrum of gram-negative aerobic pathogens. The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics.

AZACTAM For Injection is a sterile, nonpyrogenic, sodium-free, white to yellowish-white lyophilized cake, containing approximately 780 mg arginine per gram of aztreonam for intramuscular or intravenous use following constitution. Aqueous solutions of the product have a pH in the range of 4.5-7.5.

INDICATIONS AND USAGE–Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: **Urinary Tract Infections** (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca*, Citrobacter* species* and *Serratia marcescens**. **Lower Respiratory Tract Infections,** including pneumonia and bronchitis caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens**. **Septicemia** caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*, Serratia marcescens** and *Enterobacter* species. **Skin and Skin-Structure Infections,** including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Citrobacter* species*. **Intra-abdominal Infections,** including peritonitis caused by *Escherichia coli, Klebsiella* species, including *K. pneumoniae, Enterobacter* species including *E. cloacae*, Pseudomonas aeruginosa, Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**. **Gynecologic Infections,** including endometritis and pelvic cellulitis caused by *Escherichia coli, Klebsiella pneumoniae*, Enterobacter* species* including *E. cloacae**, and *Proteus mirabilis**.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy–Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organisms(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an antianaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS–Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS–Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin). Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

PRECAUTIONS–General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility–Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy–Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers–Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use–Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS–Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity; *Hypersensitivity*–anaphylaxis. *Hematologic*–pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. *Gastrointestinal*–abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). *Dermatologic*–purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. *Cardiovascular*–hypotension, transient ECG changes (ventricular bigeminy and PVC). *Respiratory*–one patient experienced flushing, chest pain, and dyspnea. *Hepatobiliary*–hepatitis, jaundice. *Nervous System*–seizure, confusion, vertigo, paresthesia, insomnia, dizziness. *Musculoskeletal*–muscular aches. *Special Senses*–tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. *Other*–vaginal candidiasis, vaginitis, breast tenderness. *Body as a Whole*–weakness, headache, fever, malaise.

Adverse Laboratory Changes–Those reported without regard to drug relationship during clinical trials were: *Hepatic*–elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*–increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Renal*–increases in serum creatinine.

OVERDOSAGE–If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION–Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg,** or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231C)

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

References: ***1.*** *Greenberg RN, Reilly PM, Weinandt WJ, et al: Comparison trial of clindamycin with aztreonam or gentamicin in the treatment of postpartum endometritis. Clin Ther 10(1):36-39, 1987.* ***2.*** *Gibbs RS, Blanco JD, Lipscomb KA, et al: Aztreonam versus gentamicin, each with clindamycin, in the treatment of endometritis. Obstet Gynecol 65:825-829, 1985.* ***3.*** *Henry SA: Overall clinical experience with aztreonam in the treatment of obstetric-gynecologic infections. Rev Infect Dis 7(suppl 4):S703-S708, 1985.*

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Frederick P. Zuspan, *Editor in Chief*
Steven G. Gabbe, *Associate Editor*
Moon H. Kim, *Associate Editor*
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Donna Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

Publisher
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2. **Dr. Quilligan**—manuscripts from states west of Pennsylvania, West Virginia, Kentucky, Tennessee, and Mississippi; manuscripts from Hawaii, Alaska, Canada, and abroad (except England, Israel, and Italy). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. *Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.*

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Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.

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Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable, but honors degrees are not permitted); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, business and home telephone numbers, and fax number of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, business and home telephone numbers, and fax number of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single-spaced sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page and key words/phrases. On manuscript page 3, type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare

Estimating length of manuscripts

With the exception of Current Development articles, the length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

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For *color figures* submit original transparencies and two sets of unmounted prints on glossy (smooth surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The top, first author's last name, and figure number must be indicated on the front of each transparency and the back of each print.

Computer-generated figures must be legible and clearly printed in jet black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend should not appear on the figure. On the back of each figure the name of the first author and the figure number should be given and the top indicated. Three original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color figures* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color figures. The preparation and submitting of color prints should fol-

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Tables. Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Titles should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. If a table or any part thereof has been taken from copyrighted material, a footnote to the table must give full credit to the original source. Special arrangements must be made with the Editors for elaborate tables because of space limitations.

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Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

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—The original and two copies of the manuscript are submitted.
—The appropriate signed completed checklist and assignment of copyright signed by **all** authors accompany the manuscript.
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—Human experimentation has been approved by the local institution as stated in the Methods section.
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Condensation

—On a separate sheet of paper, page 2 of the manuscript, a single sentence limited to 25 words delineating the essential point(s) is typed double-spaced.

Abstract

—The abstract is typed double-spaced with required margins (on page 3) headed by the title and the author(s) name, not exceeding 150 words. For case reports and brief communications the limit of 50 words is not exceeded.
—Three to five key words or short phrases are placed beneath the abstract on page 3.

Tables

—Each table headed by a title and numbered in Roman numerals is typed double-spaced on a separate page.

—Tables are cited in sequence in the text.

Figures

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—The format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" is used. Examples shown in Information for Authors were followed.

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AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

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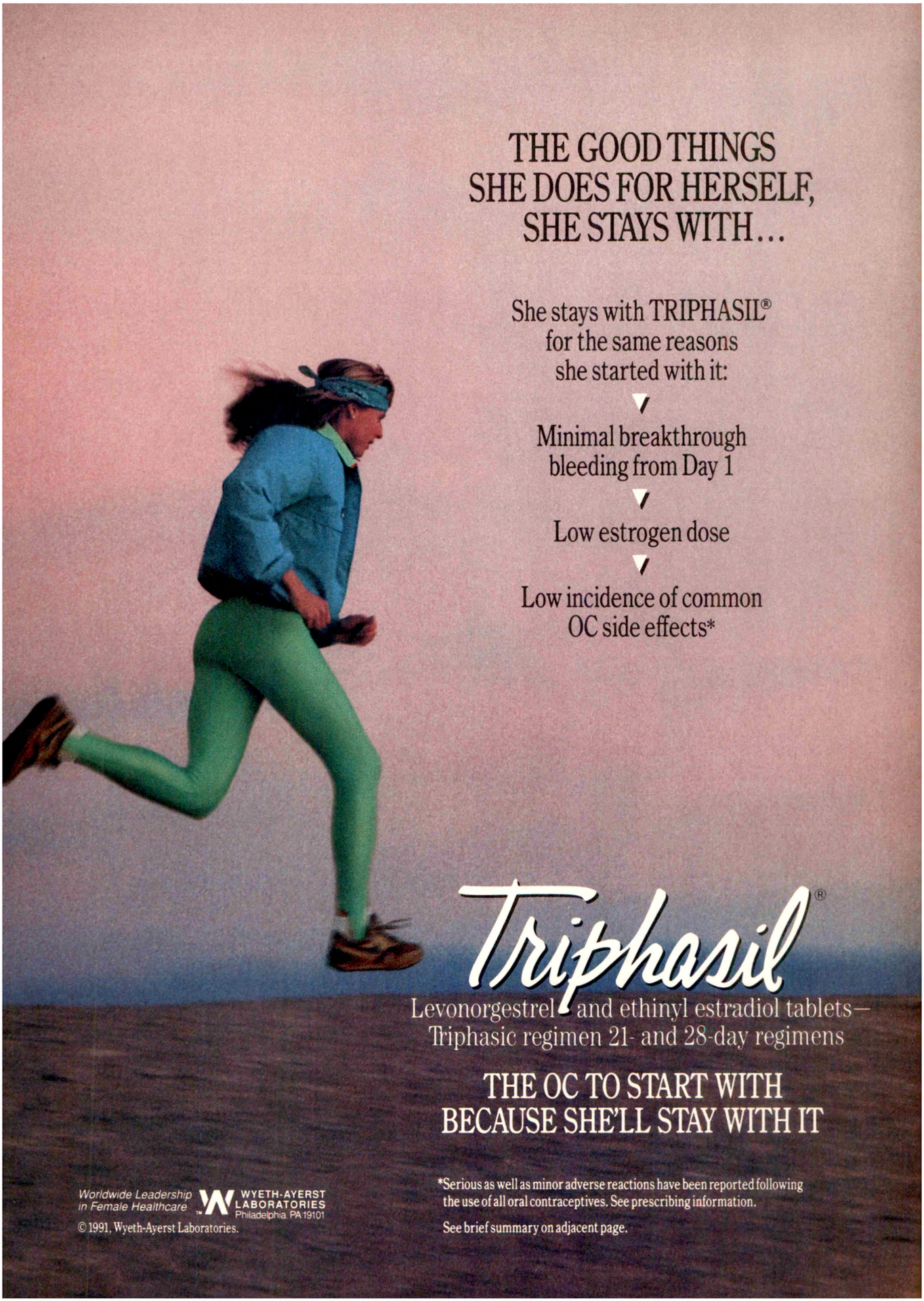
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Indications and Usage — TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications — OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thrombo-embolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners pre-scribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS — In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI) — An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE — In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES — A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34 — 5/100,000; ages 35-44 — 33/100,000; ages 45-49 — 140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS — Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions* — There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma* — Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors* — Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring* — Use of female sex hormones — both estrogenic and progestational agents — during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease* — Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects* — Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure* — Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache* — Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities* — Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy* — Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding* — OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions — GENERAL — 1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear. 2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related. 4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined. 8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's. a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient — See Patient Package Labeling.

Drug Interactions — Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis — See Warnings section for information on carcinogenesis.

Pregnancy — Category X. See Contraindications, Warnings.

Nursing Mothers — See Warnings.

Adverse Reactions — An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose — Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration — For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days. For full details on dosage and administration see prescribing information in package insert.

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CLINICAL SECTION

Clinical Opinion

Fertility retention in the patient with genital prolapse

David H. Nichols, MD

Providence, Rhode Island

There are several treatments from which to choose for the patient with symptomatic uterine prolapse who wishes to retain fertility. Transabdominal construction of a sacrocervical "ligament" with transplanted fascia lata femoris is a useful solution to this problem. The technique is described. (AM J OBSTET GYNECOL 1991;164:1155-8.)

Key words: Sacrocervicopexy, genital prolapse, sacrocervical ligament construction

From time to time one will see a patient with symptomatic uterine prolapse, usually parous, who wishes relief of symptoms and also wishes to retain the uterus and fertility. It is possible to assemble a spectrum of care from which a proper choice for this patient may be made.

History and diagnosis

It is important to try to establish the cause of the prolapse if possible. Usually it will be related to trauma from chronic respiratory disease, a lifestyle of heavy lifting, or previous obstetric damage. In some patients there may be a family history of genital prolapse, which suggests a congenital anomaly in the architecture of the pelvis itself, the elasticity of the patient's pelvic connective tissues, or a disturbance in the innervation of the voluntary muscles. The onset of the problem is often abrupt, the progress usually rapid, and it may be seen during the course of the previous pregnancy or immediately post partum.

The more common type of prolapse is the so-called uterovaginal or sliding prolapse that relates to damage of the connective supporting tissues of the uterus, cervix, and upper vagina. There may be a history of preexisting uterine retroversion that may coincide with a defect in the vaginal axis, which may be oriented in a more or less vertical plane. In this circumstance the cervical and vaginal portions of the cardinal and uterosacral ligament complex have become stretched, permitting these structures to sag demonstrably and progressively without coincident major damage to the pelvic and urogenital diaphragm (Fig. 1). A less common type is that represented by a general prolapse in which there is failure of all the pelvic supporting systems including those of the upper vagina as well as the urogenital and pelvic diaphragm including the levator ani. This may be of a congenital-type basis as seen in the younger age category of prolapse patients. The latter type is the more ominous prognostically because there is little in the way of gynecologic soft tissue strength to which to attach the vault of the vagina with the anticipation that it will remain there. With both types, coincident damage to the broad ligament and round ligaments will permit coincident retroversion, which stretches the ovarian ligament and usually the infundibulopelvic as well.

The patient should be examined first in the lithotomy position, where a bimanual examination will confirm the size, shape, and position of the uterus and adnexa. The patient is examined first at rest, and then during a Valsalva maneuver. Any exterior prolapse is replaced within the vagina, and without touching the patient she is asked to strain again by a Valsalva maneuver to de-

From the Department of Obstetrics and Gynecology, Brown University Program in Medicine.

Reprint requests: David H. Nichols, MD, Center for Women's Surgery, 100 Dudley St., Providence, RI 02905-2401.

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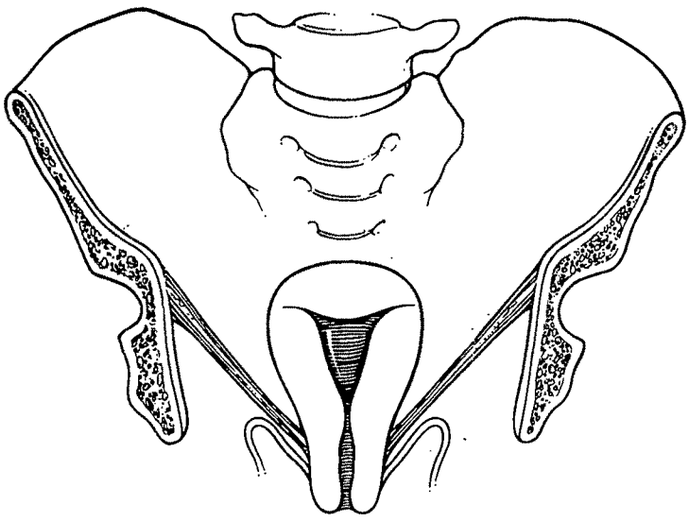


Fig. 1. Uterovaginal prolapse is shown in standing patient. Usually enterocele is present, cervix tends to be elongated, but rectocele is not necessarily present.

termine what part of the vagina appears first, as suggested by Bonney.[1] The appearance first of the vaginal vault or cervix followed by a cystocele and rectocele indicates a site of primary damage to the upper suspensory system (cardinal and uterosacral ligament complex). Initial appearance of a cystocele and rectocele followed by the cervix or vaginal vault indicates a site of primary damage to the lower supporting system (pelvic and urogenital diaphragms). This information is useful in implementing Bonney's precept: if surgical success is to be optimal, the surgeon should recognize the site of primary damage, which should be overrepaired.

The surgeon should now examine the patient's ability to contract voluntarily her pubococcygei and external anal sphincter, which will correlate to some measure with the effectiveness of innervation of these muscles by the pudendal nerve. Inability to contract them, particularly when associated with a history of rectal and urinary incontinence, strongly suggests the presence of a pudendal neuropathy. The surgeon should observe the level of the patient's perineum in relation to her glutei, inasmuch as a pudendal neuropathy is an etiologic factor for perineal prolapse. A perineal descent may indicate a major defect of the levator ani, which if symptomatic requires a separate surgical repair such as a retrorectal levatorplasty[2] or the Parks postanal repair. The presence of a rectal prolapse should be noted as an actual intussusception of the bowel. One should ask the patient to cough to determine whether there is any loss of urine. Careful inspection is made as to the support of the anterior vaginal fornix, and whether the fornix is elevated perceptably when the patient voluntarily contracts her pubococcygei, as a means of evaluating any coincident paravaginal defect.

Pelvic examination when the patient is standing (first while at rest, then when "holding" or contracting her pelvic muscles, and then when straining) will give the additional evidence of the effect of gravity on the patient's prolapse (Fig. 2). The patient is then returned to the examination table and the third pelvic examination is performed while she is in the lithotomy position and a tenaculum or long Allis clamp is applied to the posterior lip of the cervix and traction is made. By palpating the location of the cul-de-sac of Douglas and the length and strength of the uterosacral ligaments, the surgeon can identify the length of the patient's cervix. Significant elongation of both cervix and strong uterosacral ligaments suggests the more frequently seen uterovaginal or sliding prolapse, whereas a short cervix, barely perceptible uterosacral ligaments, and no enterocele suggest the less common but prognostically ominous general prolapse.

Treatment

Nonsurgical. If the pubococcygei are strong and the cervix is not elongated, one can try temporary treatment with a pessary, especially of the plastic ring-type, accompanied by a program of vigorous Kegel perineal resistive exercises (i.e., 15 voluntary squeezes six times a day). If the patient can retain the pessary she should be urged to complete her childbearing and then plan for a vaginal hysterectomy with colporrhaphy and probable McCall or New Orleans-type culdoplasty. If

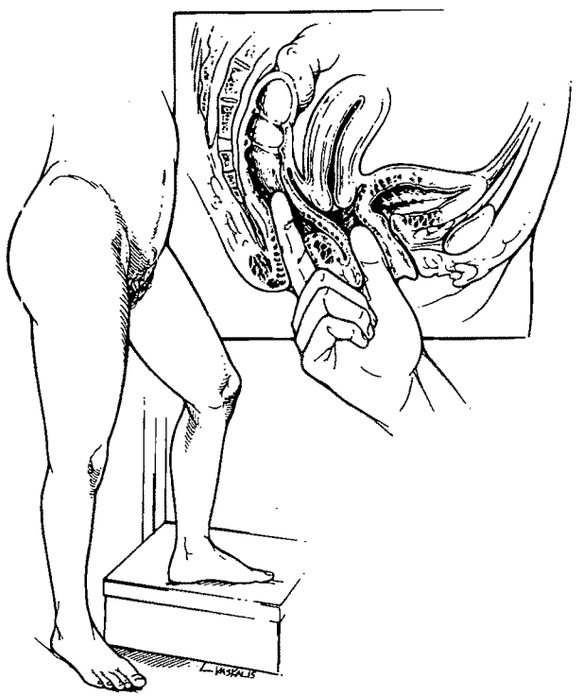


Fig. 2. Examination of patient while she is standing permits evaluation of presence or absence of prolapse of vaginal vault, extent of cystocele both at rest and when the patient strains, presence of any perineal defect, low, mid or high rectocele, and by asking patient to strain as by Valsalva maneuver, presence of palpable enterocele.

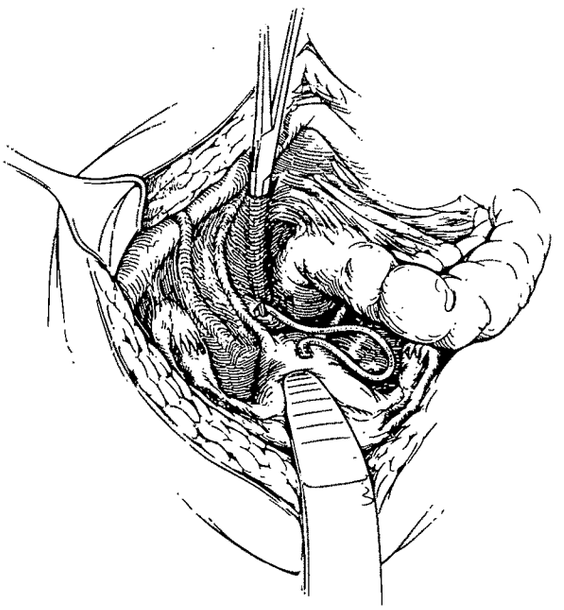


Fig. 3. Abdomen is opened, and bowel content of pelvis gently packed away and midline incision is made through peritoneum in upper third of hollow of sacrum, carefully avoiding sacral blood vessels. Retroperitoneal tunnel is established by blunt dissection from this incision beneath cul-de-sac of Douglas to site of junction of uterosacral ligaments with posterior cervix. Randall-type kidneystone forceps is useful in leading strip of fascia lata or plastic fabric through tunnel, where it is carefully fixed, first to posterior portion of cervix at its junction with uterosacral ligaments.

the pubococcygei are demonstrably weak or if the patient cannot hold a pessary, then the patient and gynecologist should consider a surgical approach to care.

Surgical

Transvaginal. If the cervix is long and there are strong but elongated cardinal and uterosacral ligaments, the patient would be a candidate for a Manchester-type repair, which includes cervical amputation, colporrhaphy, and crossing of the stumps of the cardinal and uterosacral ligaments, which are fixed to the anterior portion of the remaining cervix. Cervical amputation scrupulously avoids the internal cervix, lest a subsequent cervical incompetency be induced. Other potential disadvantages of the Manchester repair include an increased risk of premature labor, soft tissue dystocia from reduction of the size of the vagina with the colporrhaphy, and a postpartum recurrence of a cystocele and rectocele from stretching these tissues beyond their limits to elasticity.

Another transvaginal approach could be that of sacrospinous cervicopexy in which the cervix at the point to which the uterosacral ligaments are attached is sewn on one side of the pelvis to the sacrospinous ligament and coccygeus muscle complex. A nonabsorbable suture will probably be chosen, but data are lacking on the effects of subsequent pregnancy on a prolapse treated in this fashion. Williams[5] described an operation of shortening the cardinal and uterosacral ligaments for a patient without marked cervical elongation, and crossing these ligaments in front of the cervix. But this operation does not appear to be widely used.

Transabdominal surgical procedures. Construction of a sacrocervical ligament without colporrhaphy and without round ligament plication[4] is recommended for this problem. Subsequent pregnancy, labor, and delivery have been uncomplicated by the procedure and there has been no recurrence of the uterine prolapse. There should be time planned for a 6- to 8-week postoperative convalescence. Through a generous lower abdominal midline incision a retroperitoneal tunnel is established or a peritoneal incision made between the upper third of the hollow of the sacrum, beneath the cul-de-sac of Douglas, to the cervix at the point where it is attached to the uterosacral ligaments. A strap or "ligament" of fascia lata femoris is attached to these structures (Fig. 3) after the cervix is manually placed into the hollow

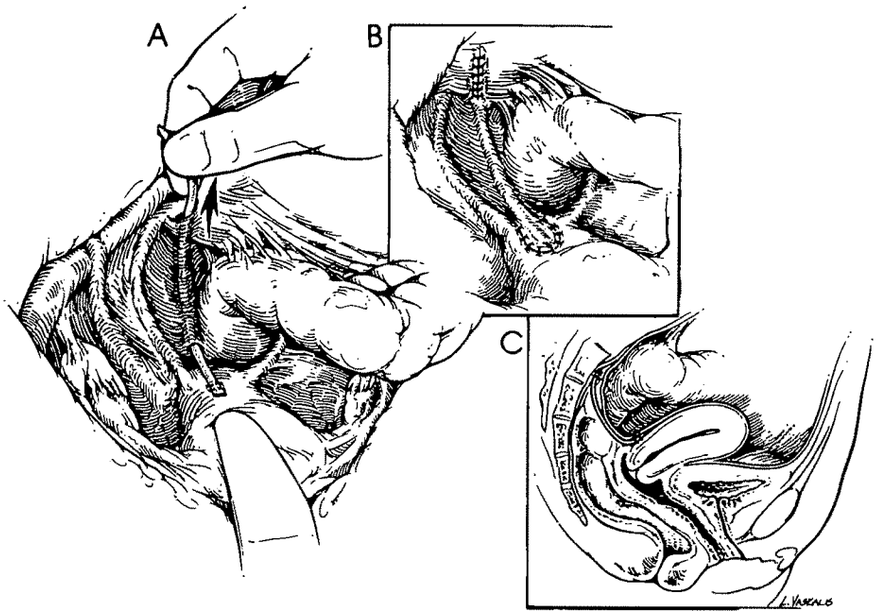


Fig. 4. A, Traction takes up some of slack in "ligament." **B**, It is fixed to periosteum of sacrum in upper third of hollow of sacrum. Some slack is made in "ligament" to permit degree of cervical mobility, and peritoneum is reapproximated from side-to-side covering new midline "ligament." **C**, End result of cervical sacropexy is shown in sagittal section. Note midline retroperitoneal ligament between posterior lip of cervix and promontory of sacrum.

of the sacrum. This will antevert the uterus. Each end of the strap is firmly sewn in place, leaving a little slack in the "ligament," and the peritoneum is reapproximated covering the ligament throughout its length (Fig. 4). By thoughtful discussion, the patient is informed preoperatively that no coincident colporrhaphy is planned, lest the vagina be made too small for future vaginal delivery and become a source of dystocia. The patient should be told that at some future date the other elements of her prolapse (cystocele, rectocele, perineal defect) will likely give evidence of progression and become the subject of a future surgical reconstruction, although by that time the patient likely will have completed her childbearing. If there is no obvious contraction of the bony pelvis, this patient should anticipate a normal vaginal delivery without recurrence of the cervical descent during the process of parturition.

The disadvantages of the operation are that the cystocele and rectocele may slowly progress in size, and if symptomatic require colporrhaphy in the future. If future hysterectomy is necessary, it may have to be performed transabdominally because of the lack of descent of the vaginal vault and cervix with traction. For the patient with symptomatic genital prolapse and urinary stress incontinence who wishes both elements to be treated with preservation of fertility, Nesbitt[5] developed a combined vaginoabdominal operation in which the paraurethral tissues of the vesical neck and the paracervical tissues of the uterine isthmus, when strong, are mobilized transvaginally, and then through a suprapubic incision are sewn to the conjoined tendon (Cooper's ligament) on each side, after any enlargement of the cul-de-sac of Douglas has been reduced by obliteration. This operation preserves a functional uterus, but to date the response to subsequent pregnancy has not been reported.

It is important to explain all treatment options to the patient, make a recommendation to her, and provide the reasons for your choice. If she agrees, supply the patient with an informed consent including the risk of recurrence and of future more complicated pelvic surgery.

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The contributions of the nurse and the machine in home uterine activity monitoring systems

Ruth B. Merkatz, PhD, RN, and Irwin R. Merkatz, MD
Bronx, New York

The relative contributions of home tokodynamometry and daily nursing telephone contact to the success of preterm birth prevention programs remains a subject of debate. Because investigators have obtained conflicting data, a reinterpretation of published results was undertaken by proposing a dynamic interface between the nurse and the machine. Experience gained from the technology and the development of nursing expertise with assessment of patient symptoms are presented as interdependent factors, both of which are critical to a therapeutic nurse-patient relationship. It is proposed that this combined interactive expertise increases sensitivity to the early recognition of preterm labor. The nurse's role in providing social support to high-risk pregnant women is then identified as a potential additional independent contributing factor to reported observed reductions in preterm births. Discussion focuses on future research, public policy issues, and the need for expanding nurse-patient interactions into the home. (AM J OBSTET GYNECOL 1991;164:1159-62.)

Key words: Home uterine activity monitoring, tokodynamometry, preterm labor, preterm birth

Preterm birth prevention programs encompassing broad-based patient and professional education are considered philosophically sound from the perspective of actively involving both pregnant women and their providers in a connected system of care.[1] Recently, such efforts have been augmented for selected women who are at high risk for preterm labor by use of programs of home uterine activity monitoring with a daily telephone contact initiated by a nurse.[2,3] Although growing consensus exists with respect to the efficacy of this combined system, debate remains centered over the relative contribution of electronic monitoring versus nursing contact.[4] For example, although Iams et al.[5] achieved a statistically significant reduction in preterm birth with implementation of such a program, they could not demonstrate a difference between a subgroup of women who obtained intensive preterm labor education, had daily nursing contact, and used the monitor when compared with a group who obtained the same education and had frequent nursing contact but who practiced twice-daily uterine self-palpation. The results of this study and other similar but as yet unpublished reports have contributed to a recent ACOG (American College of Obstetricians and Gynecologists) committee opinion statement expressing reluctance to recommend home uterine activity monitoring devices for routine clinical use.[6] The ACOG committee concluded that further prospective controlled studies must still precede public policy decisions with respect to widespread implementation. Nevertheless, it is our opinion that important insights can be gained from a reinterpretation of already published results. We propose a theoretical dynamic interface among nurse, machine, and patient as one means of reconciling previously conflicting reports.

From the Program to Reduce Obstetrical Problems and Prematurity in the Bronx (PROPP), Department of Obstetrics and Gynecology, Albert Einstein College of Medicine.
Reprint requests: Ruth B. Merkatz, PhD, RN, Department of Obstetrics and Gynecology, Albert Einstein College of Medicine, 1300 Morris Park Ave., B-501, Bronx, NY 10461.
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The nursing component in clinical studies of home uterine activity monitoring

The results of the Iams study have been interpreted to suggest that the home uterine activity monitoring failed to independently add value to the nurse-initiated telephone contact.[5] Several relevant questions exist with respect to the influence of the study's design on this conclusion. Most important, the authors themselves suggest the possibility of a Hawthorne effect; i.e., that the knowledge that a study was being conducted was, in itself, a factor that created change. This issue was raised because the same nurses cared for both groups of pregnant women enrolled in the study. The design created an opportunity for both the monitored women and the control women to respond in accordance with similar nursing expectations. It also increased the nurses' sensitivity toward recognition of patients' signs and symptoms of early preterm labor, regardless of whether a monitor was used. From a theoretical nursing perspective, it may be assumed that the critical thera-

peutic relationship between patient and nurse was established without respect to group assignment.

The value of a growing clinical expertise gained by participating nurses over the 2½ years of the study is an important factor in consideration of the extent of the Hawthorne effect. This consideration may help to explain how women in the Iams study who did not use the uterine monitor were also able to achieve important reductions in preterm births.[5] A parallel illustration that is related to the development of skills and expertise is provided by the Dreyfus Model of Skill Acquisition.[7] This model has been used to explain how pilots develop the knowledge necessary to safely guide their planes. The expert pilot, in contrast to the novice pilot, makes decisions that are based on a transformation of knowledge rather than the application of the fixed rules used during learning stages. With experience, the expert pilot is able to deviate in the manner in which he or she utilizes the information displayed on the plane's video panel. A similar opportunity exists for nurses who work with women at risk for preterm birth and who use the home uterine activity monitoring systems. The nurses interpret uterine monitoring strips on a regular basis over an extended period of time and then discuss their findings about specific symptoms with the respective patients. These nurses progressively gain an expertise that enables them to extrapolate these daily experiences to other related clinical situations. They become increasingly skilled in deciphering their patients' reports of symptoms without depending as much on viewing transmitted uterine activity strips. Thus in Iams' study it can be suggested that nursing expertise in combination with technology served as an important method of assessing women in both subgroups of patients and that this expertise may well have served as a confounding variable. It is also plausible to assume that the nurses' recognition of the clinical signs and symptoms of preterm labor in both groups of pregnant women also improved over time. This possibility may provide the needed explanation for the observed decline in preterm births evidenced for all women during the second year of the investigation. It appears that the two groups of subjects ultimately may have been exposed to the same clinical nursing intervention that improved progressively as a result of a growing expertise among the nurses. The technology, on the other hand, was fundamental in contributing to the development of this nursing expertise.

A study reported by Morrison et al.[3] did result in a lower preterm delivery rate among pregnant women who were monitored, when compared with control women who used uterine self-palpation. In this instance, however, the same group of nurses was not involved in both arms of the investigation, and expert nursing skills specific to recognizing uterine activity were probably not applied uniformly to all subjects (Morrison JC. Personal communication, December, 1989). As a result, an "independent" contribution of the monitoring system may have been able to surface to the extent that these investigators could have achieved statistically significant outcome differences between the two groups of subjects. Their finding of a significantly earlier identification of preterm labor in the monitored group is consistent with such an assumption. The authors were able to conclude that objective information obtained from home uterine activity monitoring did indeed augment the nursing contribution in reducing preterm deliveries in a monitored group of patients.

The nurse-patient relationship in association with uterine monitoring systems

The issue of using uterine self-palpation as a control in both the Iams and Morrison studies raises additional theoretical questions with respect to the nurse-patient-physician relationship.[3, 5] The practicality and validity of self-palpation for the presence of uterine activity associated with early preterm labor has recently been challenged. Newman et al.[8] showed that patient perception of uterine activity failed to yield consistent, reliable information. Brustman et al.[9] confirmed this finding and demonstrated a lack of improvement with intensive nursing educational support. These investigators have hypothesized a physiologic explanation for the attenuated perception of preterm uterine activity. If such a phenomenon is indeed operative, it would appear that asking women to engage in twice-daily uterine self-palpation has inherent potential problems. When women are asked by their providers of care to participate in time-consuming health-related activities for which there is minimal benefit, they may ultimately discontinue the prescribed regimen. Both the nurse-patient relationship and the physician-patient relationship will thereby be threatened. This notion is supported by a finding in the Iams study of a significantly higher drop-out rate among those women who used uterine self-palpation when compared with those who used electronic monitoring (21% vs 10.2%).

Requesting women to invest their time in self-monitoring of uterine activity raises an important issue of patient "honesty" associated with the reporting process. Experience documented with patient self-reporting of blood glucose values suggests that this issue also has the potential to exert a negative influence on the critical therapeutic relationships between providers and patients. Traditional logbook reporting of blood glucose values by both pregnant and nonpregnant diabetic patients proved to be unreliable when validated by a memory-based glucometer system.[10] "Phantom" values that were substituted for testing

omissions and were lower than actual blood glucose values were common patterns in patients' logbook recordings. A significant improvement was achieved only after the patients were informed about the memory chip in the glucometer and were made aware that the true glucose values were retrievable. Physicians, diabetic nurse educators, and their patients could then work together to achieve better glucose control on the basis of real data and an improved relationship of mutual trust. The potential analogy with the two choices of method for patient monitoring of preterm labor is striking. The technique of uterine self-palpation may be difficult to perform because perception of preterm uterine activity that occurs before actual labor may be attenuated. Contractions are usually painless and often can be difficult to distinguish from fetal movements. Furthermore, the mother receives no external verification of her perceptions. In contrast, when a monitor is utilized, the patient receives both feedback and positive reinforcement on a more objective basis. Although her perceptions may or may not be verified by the tracing, the feedback received may help her to distinguish contractions from other sensations. In addition, decisions regarding activity, medications, or the need for physician notification can be jointly determined by the patient and the nurse. Thus the total onus for accurate identification of early preterm labor is no longer the sole responsibility of the pregnant woman.

It could be argued, on the other hand, that the electronic monitoring system forces the mother to become unduly dependent on the nurse for feedback and reinforcement. A system of home uterine activity monitoring that allows pregnant women to see their own recordings at home provides a potential solution to this theoretical problem. This approach offers women the opportunity to improve their own skills and to verify perceptions while maintaining important contact with the nurse. The nurse-patient relationship can ultimately be strengthened by such an interactive system.

Social support provided by the nurse and its influence on preterm labor

It has been estimated that as much as 40% of low birth weight can be traced to environmental and psychosocial factors.[11] In this context, an additional explanation for the more favorable outcomes achieved by most home monitoring initiatives is the contribution of increased social support provided by the nurses. It has been hypothesized that patient stress and anxiety are important underlying variables in preterm labor and that stress and anxiety are presumably mediated by catecholamine release, changes in uterine blood flow, or other physiologic processes. It has been suggested that in pregnancy, as in other health-related situations, perceived social support can serve to buffer the adverse physiologic effects of stress.[11-14] Social support may also exert a positive influence on the pregnant woman's own health-seeking behaviors.[13-15] The provision of added social support in pregnancy has therefore been considered a strategic intervention in reducing the incidence of prematurity.[13, 15]

To date, attempts to augment social support for improving perinatal outcome have yielded conflicting results. In the United States, programs by Heins et al.[15] and Olds et al.[16] have resulted in significant reductions in preterm birth. These two initiatives have focused on providing social support to low-socioeconomic, pregnant teenagers via either specially trained "resource mothers" or nurses who make home visits. On the other hand, Spencer et al.,[17] in Manchester, England, did not achieve similar benefits by using "family workers" to visit low-income women who were also at high risk for preterm birth. These investigators acknowledge that a high incidence of "refusers" after randomization may have affected their study negatively. The fact that 58.7% of the women in their experimental group chose not to accept home visitation underscores the importance of carefully structuring interventions to suit the specific needs of targeted women. The majority of refusers in the Manchester study claimed that they already had adequate social support. A current model suggests that social support is an interactive process in which the recipient must play an active role.[18] In such a theoretical model the pregnant woman must not only desire the intervention but she must not be made to feel threatened by its potential intrusiveness.

Although there is no consistent definition of social support, most investigators concur that it generally includes interactions that provide recipients with a sense of security, a reassurance of self-worth, and an opportunity to obtain valued objective information.[18] Daily telephone contact between a knowledgeable nurse and the patient at risk for preterm birth may facilitate such interactions. Such contact represents a relatively nonintrusive means of providing support while focusing primarily on patient-driven specific issues. It may be presumed therefore that such support was provided to the largely middle-class sample of women who constituted Iams' study group and that this type of support could have contributed to favorable outcomes in these patients. An opportunity for the pregnant woman to converse daily with a concerned provider is difficult to achieve in traditional private-practice settings. Patients are typically reluctant to make repeated telephone calls for fear of imposing excessively on a busy office staff.

Future research issues

Preterm birth disproportionately affects poor, minority women who are primarily cared for in clinic settings.[11, 19] For such women social disorganization and

hostile environmental conditions often create unusually stressful life circumstances. Theoretically, these women should also benefit from the focused social support embodied in the home uterine activity monitoring systems and from the expertise of the nurses. Studies of efficacy in this high-risk group have been limited, however, because of access to care and other public policy issues. Even access to telephones may represent a limiting factor for this research. The study of Morrison et al.[3] is of particular importance because of its demonstrated benefits in the early diagnosis of preterm labor among black women in rural Mississippi. A more recent collaborative trial from four tertiary centers, including two with a large population of indigent black women, provides similar encouraging findings.[20] Future studies concentrating on efficacy questions among different groups of high-risk women are clearly indicated. Such studies must be afforded a fair priority status vis-à-vis the multiple competing demands for limited resources.

Future prematurity prevention initiatives incorporating home uterine activity monitoring or other technologic interventions should also stress the importance of including periodic face-to-face contacts between patient and nurse. One potential criticism of machine-driven forms of nursing practice has been a concern that the nurse might become too technologically dependent and might lose her holistic orientation.[21] Home visitation to pregnant women is a strategy that is used widely in other Western countries but remains uncommon in the United States. Research with respect to monitoring uterine activity should be broadened to include this perspective. These comprehensive programs have the potential to further our growing national commitment toward providing improved care for pregnant women. Physicians and nurses must continue to exert a leadership influence through their interdisciplinary collaborations.

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Bleeding patterns during continuous combined estrogen-progestogen therapy

Ulla Marslew, MD, Bente J. Riis, MD, and Claus Christiansen, MD
Glostrup, Denmark

Bleeding and climacteric symptoms were recorded in two groups of postmenopausal women receiving either continuous combined estradiol and norethisterone acetate or estradiol and cyproterone acetate. Out of a sample of 99 postmenopausal women aged 45 to 54 years, 86 completed a 2-year, double-blind, placebo-controlled study. Comparison of the bleeding patterns in the two groups revealed a statistically significant difference: More women in the estradiol–cyproterone acetate group experienced bleeding and for a longer duration. Thirteen women in the estradiol–norethisterone acetate group were amenorrheic, compared with two in the other group. The Kupperman index score in both groups declined to about 30% to 40% of initial values ($p < 0.001$). The hot flushes in both treatment groups decreased to a highly significant degree ($p < 0.001$), to a value below 20% of baseline values. We conclude that a continuous combination of estrogen and progestogen can produce amenorrhea and symptomatic relief. However, the progestogen components seem to differ in their ability to control bleeding. (Am J Obstet Gynecol 1991;164:1163-8.)

Key words: Continuous estrogen-progestogen, bleeding pattern, climacteric symptoms

Postmenopausal hormone replacement therapy is widely prescribed to alleviate postmenopausal symptoms and to prevent osteoporosis.[1, 2] For women with an intact uterus, the hormone replacement therapy should consist of estrogen combined with a progestogen to eliminate the increased risk of endometrial hyperplasia and cancer.[3, 4] To imitate the normal menstrual cycle, the progestogen is generally added sequentially. However, this therapy has the major disadvantage of inducing bleeding.

Continuous combined estrogen-progestogen therapy was introduced to overcome the problem, the philosophy being that continuous progestogen administration would provide a continuous inhibition of the estrogenic action and thereby keep the endometrium atrophic. The risk of hyperplasia would thus be eliminated and women would be spared the inconvenience of bleeding.

The aim of this 2-year, double-blind, placebo-controlled study was to compare the bleeding pattern during two continuous combined regimens. The changes in menopausal symptoms were also recorded and compared.

From the Department of Clinical Chemistry, Glostrup Hospital, University of Copenhagen.
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Reprint requests: Dr. Ulla Marslew, Department of Clinical Chemistry, Glostrup Hospital, University of Copenhagen, DK-2600 Glostrup, Denmark.
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Material and methods

The participants were recruited in 1983[5, 6] and 1988.[7] Ninety-nine women aged 45 to 54 years were selected by postmenopausal age (6 months to 3 years) and freedom from past and present diseases and medications known to influence the variables studied or to contraindicate the medication used in the trial. Further details of the selection procedure are given elsewhere.[5, 7] After the initial examination the women were randomly allocated to two groups. Group 1 received continuous estradiol–norethisterone acetate (2 mg of 17β-estradiol and 1 mg of norethisterone acetate) ($n = 24$) or placebo tablets that were identical in appearance ($n = 25$).[5, 6] Group 2 received continuous estradiol–cyproterone acetate (2 mg of estradiol valerate and 1 mg of cyproterone acetate) ($n = 25$) or placebo tablets that were identical in appearance ($n = 25$).[7]

All women gave informed consent (Helsinki Declaration II) and the trials were approved by the Copenhagen County Ethical Committee. The women were treated for 2 years with an examination done every 3 months.

Table I. Clinical data and initial values of serum estradiol in pooled treatment and placebo groups (mean ± SEM)

	No.	*Age (yr)*	*Postmenopausal age (mo)*	*Height (cm)*	*Weight (kg)*	*Kupperman index**	*Serum estradiol† (nmol/L)*
Estradiol-norethisterone acetate	43	50.7 ± 0.3	25.2 ± 1.7‡	162 ± 1	69.2 ± 1.5	12 (4-23)	0.109 (0.093-0.127)
Estradiol-cyproterone acetate	43	50.8 ± 0.3	19.3 ± 1.4	164 ± 1	65.7 ± 1.5	11 (4-29)	0.087 (0.077-0.098)

*Mean and range.
†Geometric mean.
‡$p < 0.01$.

Between 21 and 24 months of therapy the women in group 2 and the corresponding placebo group had an endometrial biopsy taken, and therefore the bleeding records of both groups at 24 months were not included in the calculations.

Compliance. Of the 49 women in group 1, 43 (88%) completed the 24-month study. Three women left the hormone group—one because of persistent uterine bleeding and two because of lack of time. Three women left the placebo group—two because of major climacteric symptoms and one because of edema of the legs and fingers. Thus 21 women remained in the hormone group and 22 in the placebo group of group 1.

Of the 50 women in group 2, 43 (86%) completed the 24-month study. Six women left the hormone group—three because of persistent uterine bleeding, two because of lack of time, and one because of edema of the legs. One woman left the placebo group because of the development of thyrotoxicosis. Thus 19 women remained in the hormone group and 24 in the placebo group of group 2.

Record of bleeding pattern. At baseline the women were shown how to record bleeding on a calendar. Each day there were asked to note down the days in which they experienced bleeding, defined as reddish vaginal discharge necessitating use of pads. At each examination done every 3 months, the women returned the completed calendar to the department and received a new one for the next 3 months.

Record of menopausal symptoms. At baseline the women were shown how to record clinical symptoms on a questionnaire, which they filled in every 3 months. The questionnaire covered the 11 symptoms on the menopausal index of Kupperman et al.[8]: hot flushes (with and without sweating), paresthesias, insomnia, nervousness, melancholia, vertigo, fatigue, arthralgias or myalgias, headache, palpitations, and formications. Each symptom on the Kupperman index was rated on a scale from 0 to 3 for no, slight, moderate, and severe complaints. To calculate the Kupperman index, the symptoms were weighted as follows: hot flushes (×4), paresthesias (×2), insomnia (×2), nervousness, (×2), and all other symptoms (×1). The highest potential score is thus 51.

Each woman was carefully instructed on how to record episodes of bleeding on a calendar and how to fill in a questionnaire on menopausal symptoms. A doctor was available to help with the questionnaire. Returned questionnaires were examined for errors.

Serum estradiol measurements. The serum concentrations of estradiol were measured every 6 months with antiserum supplied by BioMarkor, and standards were prepared with estradiol supplied by Sigma. The intraassay and interassay variations were 4% and 14%, respectively, and the sensitivity was 0.010 nmol/L.

Statistical analysis. Student's *t* test for unpaired data was used to test the significance of differences in all the initial values (Table I), except the initial Kupperman index, which was tested by Wilcoxon's test for unpaired data.

The pretreatment values of the menopausal index and the hot flushes for each subject were assigned a value of 100%, and subsequent measurements were expressed as percentages of the value. To eliminate the genuine climacteric complaints also experienced by premenopausal women,[9] women with an initial Kupperman index score of <4 were not included in the calculations of the Kupperman index. Student's *t* test for paired data was used to test the significance of differences within the groups and the *t* test for unpaired data was used to test the significance of differences between the groups.

The frequency of bleeding during each 3-month period was calculated as the number of women who experienced bleeding, irrespective of the number of episodes (each woman was counted only once). The average duration (days) in each 3-month period was calculated as the sum of the days on which bleeding occurred divided by the number of women who experienced it in this period. Fisher's exact test was used to test the significance of differences between the groups.

Changes in the serum concentration of estradiol were

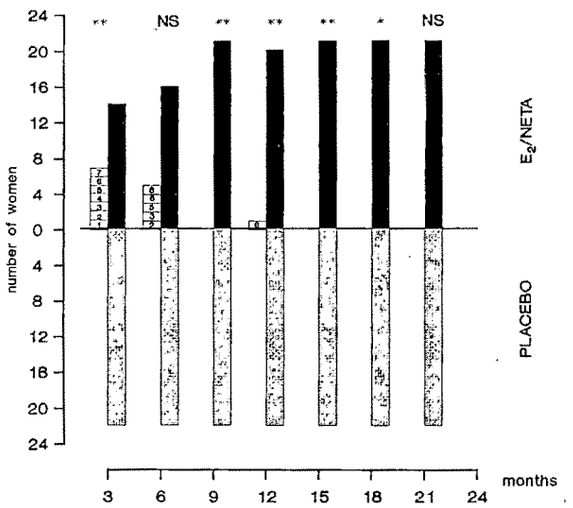


Fig. 1. Pattern of bleeding, expressed as number of women with episodes in each 3-month period—treatment with estradiol–norethisterone acetate. *Open bars* represent women with bleeding. *Hatched* and *closed bars* represent women without bleeding. *Number* or *letter* in *open bars* identifies woman. *NS,* Not significant; *asterisk,* $p = 0.05$; *double asterisk,* $p < 0.01$; these values represent difference between estradiol–norethisterone acetate and estradiol–cyproterone acetate groups.

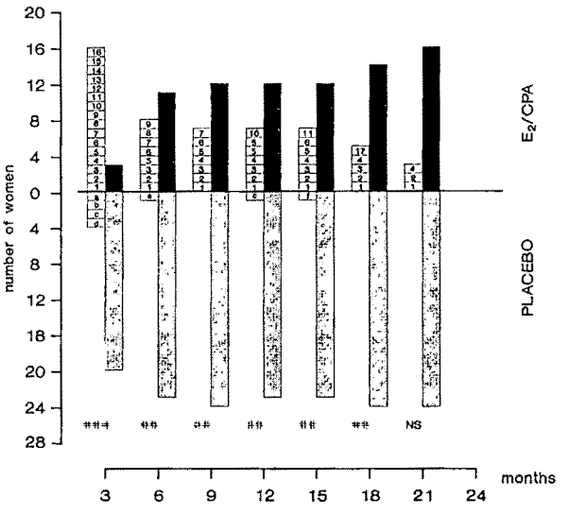


Fig. 2. Pattern of bleeding, expressed as number of women with episodes in each 3-month period—treatment with estradiol–cyproterone acetate. *Open bars* represent women with bleeding. *Hatched* and *closed bars* represent women without bleeding. *Number* or *letter* in *open bars* identifies woman. *NS,* Not significant; *double number sign,* $p < 0.01$; *triple number sign,* $p < 0.001$; these values represent difference between estradiol–cyproterone acetate group and corresponding placebo group.

calculated by the geometric mean, because of a non-normal distribution of the data.

Results

Table I gives the clinical data and the initial values of serum estradiol in the two groups of women. Group 1 women had a slightly higher postmenopausal age ($p < 0.01$).

Fig. 1 shows the pattern of bleeding in group 1. In the first 3 months bleeding occurred in seven women receiving estradiol–norethisterone acetate for an average duration of 10 days. In the next 3 months the number of women in the estradiol–norethisterone acetate group with bleeding had decreased to five, with average bleeding duration remaining at 10 days. Four of the women were among the seven with episodes in the first period, whereas one woman first experienced bleeding in the second period. After the first 6 months one woman in the hormone group experienced one further episode of bleeding (10 days). None of the women in the placebo group had bleeding.

Fig. 2 shows the pattern of bleeding in group 2 treated with estradiol–cyproterone acetate or placebo. In the first 3 months bleeding occurred in 16 women in the hormone group for an average duration of 10 days and in four women in the placebo group for an average of 7 days. In the next 3 months the number of women in the hormone group had decreased to eight, with an average duration of 15 days, and one woman in the placebo group had bleeding for 7 days. In the next three periods (15 months of treatment in all) the number of women in the hormone group with bleeding was constantly 7; the duration ranged from 7 to 10 days. Two women in the placebo group had bleeding episodes in the fourth and fifth periods, each lasting 5 days. A number of women treated with estradiol–cyproterone acetate also had bleeding in periods six and seven. As the figure shows, the same women in the hormone group tended to have repeated episodes.

The data summarized in Table II give the number of women who experienced bleeding and the average duration (days) in 3-month periods for 21 months of therapy. The number of women who dropped out of the study because of persistent uterine bleeding are also listed. The women in the estradiol–cyproterone acetate group had more bleeding episodes than those in the estradiol–norethisterone acetate group. This was statistically significant ($p < 0.01$ to 0.05). In the estradiol–norethisterone acetate group 13 women were amenorrheic throughout the study, each of four women had one episode of bleeding, three women had two episodes, and one woman had three episodes. In the estradiol–cyproterone acetate group two women were amenorrheic, each of six women had one episode of

Table II. Number of women experiencing bleeding and average duration in days in each 3-month period

Period	*Estradiol–norethisterone acetate*			*Placebo*		*Estradiol–cyproterone acetate*			*Placebo*	
	No.	*Duration (days)*	*Dropouts (No.)*	*No.*	*Duration (days)*	*No.*	*Duration (days)*	*Dropouts (No.)*	*No.*	*Duration (days)*
1	7 (33%)	10	0	0	0	16 (84%)*	10	1	4 (17%)	7
2	5 (24%)	10	1	0	0	8 (42%)†	15	2	1 (4%)	7
3	0	0	0	0	0	7 (37%)*	7	0	0	0
4	1 (5%)	10	0	0	0	7 (37%)*	10	0	1	5
5	0	0	0	0	0	7 (37%)*	10	0	1	5
6	0	0	0	0	0	5 (26%)‡	5	0	0	0
7	0	0	0	0	0	3 (16%)†	4	0	0	0

Women who dropped out because of persistent uterine bleeding are listed.

*$p < 0.01$ (difference between estradiol–norethisterone acetate and estradiol–cyproterone acetate groups).

†Not significant (difference between estradiol–norethisterone acetate and estradiol–cyproterone acetate groups).

‡$p < 0.05$ (difference between estradiol–norethisterone acetate and estradiol–cyproterone acetate groups).

bleeding, four women had two episodes, one woman had three, three women had five, one woman had six, and two women had seven episodes.

Fig. 3 shows the Kupperman index score for the 11 symptoms described in the Material and methods section. In both hormone groups the score declined to a highly significant degree during the first 3 months of treatment, by 60% and 70% of the initial values, respectively ($p < 0.001$). Both placebo groups had unchanged values.

Fig. 4 shows the hot flushes as an isolated symptom in the four groups of women. In both hormone groups the hot flushes decreased to a highly significant degree, to a value <20% of baseline ($p < 0.001$). There were no significant differences in response between the two treatment groups. The placebo groups had unchanged values.

Comment

The populations in this study were recruited in 1983 (estradiol–norethisterone acetate group) and 1988 (estradiol–cyproterone acetate group) by the same selection procedure. A questionnaire was mailed to a very large number of women, aged 45 to 54 years, living in the "catchment" area of Glostrup Hospital. This population has been shown to be representative of Danish middle-aged women.[10] The frequency of response was above 80% and our study samples may therefore be considered representative of healthy postmenopausal women.

The study design ensured a double-blind procedure and the women were examined at 3-month intervals. This afforded a continuity in the data sampling.

The estradiol–norethisterone acetate group initially had a 6-month higher postmenopausal age than the estradiol–cyproterone acetate group ($p < 0.01$). This otherwise confounding factor was corrected for by the longitudinal design.

Compliance was above 85% and, although more women in the estradiol–cyproterone acetate group left the study than in the other groups, the number ($n = 19$) completing it was still acceptable. One woman in the estradiol–norethisterone acetate group and three women in the estradiol–cyproterone acetate group left the study because of persistent uterine bleeding.

It has been suggested[11, 12] that hormone replacement therapy decreases the risk of cardiovascular diseases in postmenopausal women. Progestogens counteract the estrogenic effect on serum lipids and lipoproteins; this may be especially marked for those derived from testosterone, such as norethisterone acetate.[13, 14] Hydroxyprogesterone derivatives, such as cyproterone acetate, may possess this effect on the lipids to a lesser extent.[13-16] New combinations of estrogen and progestogen, including hydroxyprogesterone derivatives, are thus being intensively studied.

A major concern with regard to postmenopausal estrogen therapy is the risk of endometrial hyperplasia and carcinoma.[17, 18] Histologic studies[19, 20] indicate that the addition of a progestogen may minimize the risk, and this has been confirmed by a recent prospective epidemiologic study.[4] The sequential addition of a progestogen produces regular bleeding in most women, which is a major disadvantage,[21] and therapy with a continuous combination of estrogen and progestogen was introduced to overcome the problem.[22] The achievement of endometrial atrophy over time requires a delicate balance between the estrogenic and progestogenic activities on the endometrium. The hormonal effect depends on the dose, rate of absorption, and half-life of the particular estrogen and progestogen; therefore the difficulty in achieving this balance is obvious.

Earlier studies[23, 24] of a continuous combination of estradiol and norethisterone acetate have demonstrated a significantly lower bleeding frequency than

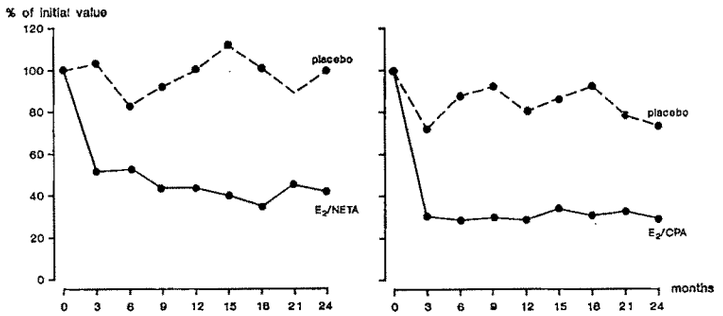


Fig. 3. Mean percentages for Kupperman index during 2 years of therapy with two continuous estrogen-progestogen treatments.

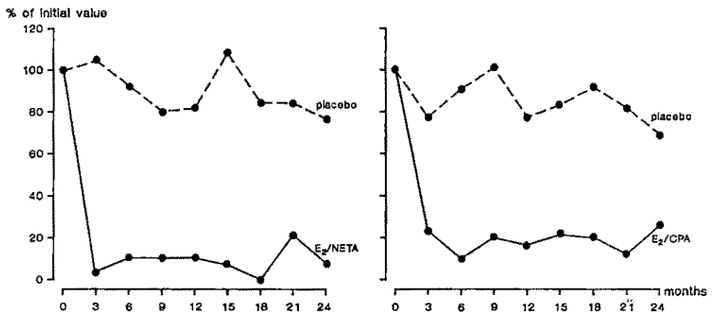


Fig. 4. Mean percentages for hot flushes (Kupperman index) during 2 years of therapy with two continuous estrogen-progestogen treatments.

that occurring with sequential hormone replacement therapy, but both studies report that a number of women (15% and 35%, respectively) experienced irregular bleeding in the first 3 months of therapy, a finding that is consistent with our results. After 3 months the frequency decreased significantly and as in our study bleeding finally stopped. In their study of four continuous estrogen-progestogen combinations (2 mg of estradiol plus 1 or 0.5 mg of norethisterone acetate or 2 mg of estradiol plus 5 or 2.5 mg of megestrol acetate), Sporrong et al.[25] concluded that a continuous combination of estradiol and progestogen can be used for long-term treatment of climacteric complaints in postmenopausal women and that after 4 months clinical efficacy of the progestogens is the same, irrespective of the type and dose administered. Their study also revealed substantially less bleeding with the formulation containing 1 mg of norethisterone acetate, whereas they had approximately the same percentage of amenorrheic women as we had in our estradiol–norethisterone acetate group and the average number of days with bleeding in their study was lower than in ours. The explanation may be that their study population was about 5 years older and thus had a correspondingly higher postmenopausal age. This may decrease the frequency of bleeding during continuous combined hormone replacement therapy, as indicated by a study from our group[26] in which 65-year-old women were given the same estradiol–norethisterone acetate combination. Bleeding was almost negligible in these women. Furthermore, the study of Hövik et al.[27] showed that when postmenopausal women were treated with continuous estradiol–norethisterone acetate immediately after sequential treatment with the same compound, the bleeding frequency was increased.

The pattern of bleeding in the estradiol–cyproterone acetate group was different from that in the estradiol–norethisterone acetate group. Significantly more women experienced bleeding during the first 6 months, and irregular bleeding continued in a substantial number of women thereafter, although it tended to decrease. This indicates that, with regard to the control of bleeding, the estrogen-progestogen balance in the estradiol–cyproterone acetate combination was inferior to that in the estradiol–norethisterone acetate combination. The half-life of norethisterone acetate is thus about 10 times lower than that of cyproterone acetate (mean ± SD, 4.2 ± 1.8 and 38 ± 14, respectively), a distinct difference because of the high affinity of cyproterone acetate to fatty tissue. This may influence the control of bleeding. On the other hand, bleeding in the estradiol–cyproterone acetate group did not result

from hyperplasia, because no endometrial biopsy specimens showed proliferation.

We conclude that a continuous estrogen-progestogen combination induces amenorrhea and affords symptomatic relief. However, the progestogens seem to differ in their interaction with estrogen to bring bleeding under control.

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Effect of low-dose aspirin on vascular refractoriness in angiotensin-sensitive primigravid women

Henk C.S. Wallenburg, MD, PhD, Guus A. Dekker, MD, PhD, Jolande W. Makovitz, MD, and Nel Rotmans, BSc

Rotterdam, The Netherlands

The hypothesis that an enhanced vasopressor response to angiotensin II in pregnancy may be corrected by suppressing production of platelet thromboxane A_2 with low-dose aspirin was tested in a randomized, placebo-controlled, double-blind trial. We studied 36 normotensive primigravid women with an elevated blood pressure response to intravenously infused angiotensin II at 28 weeks' gestation; 18 women received 60 mg of aspirin daily and the same number received matched placebo until 34 weeks' gestation, when angiotensin-sensitivity was again determined. In women taking aspirin, values of thrombin-induced platelet malondialdehyde production were approximately 10% of those determined in the placebo group, indicating marked suppression of thromboxane A_2 synthesis. In the aspirin group vascular refractoriness to angiotensin II was restored in 14 of 17 treated women, by comparison with 5 of 15 women in the placebo group who had remained normotensive. These results support the hypothesis that prostacyclin/thromboxane imbalance is an important pathophysiologic factor in the development of the enhanced angiotensin-sensitivity associated with pregnancy-induced hypertensive disorders. (AM J OBSTET GYNECOL 1991;164:1169-73.)

Key words: Angiotensin II, pressor response, low-dose aspirin, prostaglandins

Pregnancy is associated with profound physiologic alterations in vascular behavior, including vasodilatation and a marked refractoriness to the vasopressor effect of intravenously infused angiotensin II.[1] The vascular adaptations to pregnancy are thought to depend to a large extent on delicate changes in eicosanoid biosynthesis, in particular involving prostacyclin and thromboxane A_2. There is increasing evidence that in normal pregnancy these changes lead to a dominance of the biologic effects of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation produced by vascular endothelium and placenta, over the opposing effects of thromboxane A_2, a potent vasoconstrictor and stimulator of platelet aggregation, mainly produced by platelets.[1, 2]

For reasons that are not yet well understood, prostacyclin synthesis may be insufficient in some, primarily primigravid, women, and the prostacyclin-thromboxane A_2 balance may become tilted in favor of thromboxane A_2,[1-3] thus leading to the vasoconstriction and platelet activation characteristic of pregnancy-induced hypertensive disorders.[4-6] Enhanced sensitivity to the vasopressor effect of infused angiotensin II can be demonstrated long before the clinical manifestation of high blood pressure[7] and has been postulated as an important pathophysiologic mechanism in the development of pregnancy-induced hypertensive disorders and an early, if not the first, sign of a prostacyclin-thromboxane A_2 imbalance.[1, 8] We have shown that a daily dose of 60 mg aspirin (acetylsalicylic acid) prevents the development of preeclampsia in primigravid women with enhanced angiotensin-sensitivity at 28 weeks' gestation,[9] probably through selective inhibition of platelet cyclooxygenase and synthesis of thromboxane A_2, resulting in correction of an imbalance between the biologic effects of prostacyclin and thromboxane A_2.[10, 11] If enhanced angiotensin sensitivity is indeed a pathophysiologic factor in the development of pregnancy-induced hypertensive disorders caused by prostacyclin-thromboxane A_2 imbalance, low-dose aspirin would also be expected to restore, or at least improve, refractoriness to the pressor effects of angiotensin II in angiotensin-sensitive pregnant women. To test this hypothesis we conducted a randomized, placebo-controlled, double-blind trial to investigate whether a low dose of aspirin, taken daily from 28 to 34 weeks' gestation, would reduce the vasopressor response to intravenously infused angiotensin II in normotensive, angiotensin-sensitive primigravid women.

Material and methods

The trial was designed to include 36 angiotensin II-sensitive primigravid women in the early third trimester of pregnancy, 18 in the treatment group and 18 in

From the Department of Obstetrics and Gynecology, Erasmus University School of Medicine and Health Sciences.
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Reprint requests: Henk C.S. Wallenburg, MD, PhD, Professor, Department of Obstetrics and Gynecology, Erasmus University School of Medicine and Health Sciences, EE 2283, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.
6/1/27044

the placebo group. We chose this number to demonstrate statistical significance with an α-error and a β-error of 0.05, assuming the possibility to restore angiotensin-refractoriness in 85% of treated and in 40% of untreated women. The assumptions were based on the results of our previous trial with low-dose aspirin,[9] on the premise that pregnancy-induced hypertensive disorders and angiotensin II sensitivity are different manifestations of the same pathophysiologic disturbance. The end point of the study was the blood pressure response to infused angiotensin II following 6 weeks of active or placebo treatment.

To recruit the required number of angiotensin-sensitive pregnant women, healthy primigravid women with an uncomplicated pregnancy of 26 weeks' duration attending the Antenatal Clinic of the University Hospital Rotterdam-Dijkzigt were given written and oral information about the study protocol as approved by the Hospital and University Ethics Committee.

Angiotensin sensitivity test. The blood pressure response to intravenously infused angiotensin II was determined at 28 weeks' gestation in 142 women who gave informed oral consent. None of the women had a history of hypertension or cardiovascular or renal disease. The course of pregnancy had been uncomplicated in all cases, with a maximum diastolic blood pressure of ≤85 mm Hg; none of the women used any drugs except oral iron supplements, and diets were unrestricted.

The angiotensin II sensitivity test was performed as described previously.[9, 12] Briefly, an intravenous catheter was introduced into the right cubital vein for administration of angiotensin II–amide (Hypertensin, Ciba Geigy, Arnhem, The Netherlands) in 5% dextrose by means of an infusion pump. Blood pressure was measured every 3 to 5 minutes in the left upper arm with a standard mercury sphygmomanometer with the women in the left lateral recumbent position. The point of muffling of the Korotkoff sounds (phase IV) was recorded as the diastolic pressure. After a stable blood pressure and heart rate had been recorded for at least 15 minutes, infusion of angiotensin II was started at a rate of 3 ng/kg/min and increased stepwise by 1 ng/kg/min at 5-minute intervals. Three blood pressures were taken at the end of each incremental dose, and the median values were recorded. The minimum amount of angiotensin II (per kilogram per minute), which caused a rise in diastolic blood pressure of 20 mm Hg above the mean diastolic blood pressure level before the start of the infusion, was defined as the effective pressor dose.

The test was stopped after the effective pressor dose had been determined in triplicate or when infusion of 12 ng/kg/min had not caused a rise in diastolic blood pressure of 20 mm Hg. A positive angiotensin II sensitivity test was defined as a test with a median effective pressor dose of 10 ng/kg/min or less.[12] All tests were carried out by one of us (J.W.M.).

Study protocol. The 36 women with a positive angiotensin II sensitivity test were enrolled in the study at 28 weeks' gestation, immediately after the test. Each woman received a coded package with 50 tablets containing 60 mg of aspirin or matched placebo, according to a randomization list. The women were instructed to take one tablet daily at breakfast over a 6-week period, until angiotensin sensitivity was again tested; at each antenatal visit they were asked how many tablets remained. The women were asked to report the use of any other drugs and were explicitly told not to use aspirin. All women involved in the study received standard antenatal care, in which the investigators were not involved.

Angiotensin sensitivity was again determined at 34 weeks' gestation in all women who had remained normotensive (diastolic blood pressure ≤85 mm Hg); the same procedure applied at 28 weeks' pregnancy was used. After the introduction of the venous catheter, a blood sample was drawn for determination of the thrombin-induced production of malondialdehyde by platelets. Malondialdehyde is a stable by-product of platelet thromboxane synthesis[13]; its thrombin-induced production is approximately 5 nmol/10^9 platelets in the third trimester of uncomplicated pregnancy.[5] Daily ingestion of 60 mg of aspirin causes about 90% inhibition of platelet malondialdehyde synthesis,[9] which can thus be used as a sensitive index of the use of aspirin. The investigators remained unaware of the results until the end of the study when the code was broken.

The study was terminated after the second angiotensin II sensitivity test at 34 weeks' gestation. Some of the women then started taking 60 mg aspirin in an attempt to reduce the risk of developing pregnancy-induced hypertensive disorders.[9]

Statistical analysis. Differences in relative frequencies between placebo and treatment groups were analyzed with the use of Fisher's exact test. The Wilcoxon test for paired and unpaired data was used as appropriate to analyze differences in continuous variables between the two groups. A probability value of <0.05 was considered significant. Where appropriate, 95% confidence intervals were calculated.

Results

At the beginning of the study the two groups were comparable with regard to age, body weight, baseline blood pressure, and heart rate (Table I). In the aspirin

Table I. Clinical characteristics of 36 angiotensin II–sensitive primigravid women at 28 weeks' gestation

	Aspirin (n = 18)		*Placebo (n = 18)*	
	Median	*Range*	*Median*	*Range*
Age (yr)	23	18-36	24	19-34
Weight (kg)	73	55-96	72	46-88
Systolic blood pressure (mm Hg)	115	103-136	111	100-128
Diastolic blood pressure (mm Hg)	70	61-80	74	66-83
Heart rate (beats/min)	80	72-104	84	76-108

group four women smoked 10 to 20 cigarettes per day, in comparison with three women in the placebo group. The dose of aspirin at 28 weeks' gestation was 0.9 ± 0.2 mg/day/kg body weight (mean ± SD).

One woman in the aspirin group withdrew from the study immediately after randomization. The angiotensin II sensitivity test was not repeated in three women in the placebo group, because they had become hypertensive (diastolic blood pressures of 95 to 110 mm Hg) before 34 weeks' gestation; in the aspirin group all women were normotensive at 34 weeks. In the placebo group the mean (±SD) value of thrombin-induced malondialdehyde production per 10^9 platelets at 34 weeks' gestation was 4.5 (±1.0) nmol, in comparison with 0.48 (±0.2) nmol in the aspirin group ($p < 0.001$); the highest value determined in the aspirin group was 1.0 and the lowest value in the placebo group was 2.8 nmol/10^9 platelets. The mean (±SD) number of days during which medication was taken in the aspirin group (40 ± 3) was not different from that in the placebo group (41 ± 4).

The median effective pressor dose at 28 weeks' gestation was 7.5 ng/kg/min in both groups (Table II). The effective pressor dose at 34 weeks was significantly higher than at 28 weeks in the aspirin group ($p < 0.01$) but not in the placebo group (Fig. 1). Seven women in the placebo group but none in the aspirin group showed a decrease in the effective pressor dose between 28 and 34 weeks' gestation ($p < 0.01$). At 34 weeks' pregnancy 14 women in the aspirin group, as compared with five in the placebo group, showed an effective pressor dose of >10 ng/kg/min ($p = 0.02$); all these women had effective pressor doses of 11 or 12 ng/kg/min, except one woman in the aspirin group with an effective pressor dose of >12 ng/kg/min in whom the actual value was not determined. When the effective pressor dose was reached during angiotensin infusion, maternal heart rate showed a significant fall of 8 to 10 beats/min before and after treatment ($p < 0.01$); the fall in maternal heart rate was not different between groups (Table II).

Since some of the women had been taking a low dose of aspirin after 34 weeks' gestation and others had not, the outcome of pregnancy was not analyzed. All infants were live-born, and serious neonatal problems were not encountered.

Table II. Angiotensin II sensitivity before the start of treatment with aspirin or placebo at 28 weeks and at the end of treatment at 34 weeks' gestation

	Aspirin (n = 17)	*Placebo (n = 15)*
Effective pressor dose (median and range)		
At 28 wk	7.5 (6-9)	7.5 (5.5-9)
At 34 wk	11 (7->12)	8 (3.5-11)
Heart rate during effective pressor dose (beats/min) (median and range)		
At 28 wk	72 (60-92)	74 (60-96)
At 34 wk	74 (60-84)*	76 (48-98)†
No. of women with effective pressor dose > 10 ng/kg/min		
At 28 wk	0	0
At 34 wk	14	5

*Effective pressor dose reached in 13 women.
†Effective pressor dose reached in 15 women.

Comment

The values of thrombin-induced production of malondialdehyde by platelets in both groups of women indicate that they had complied with the prescribed regimen. The observation that three of 18 women in the placebo group had already become hypertensive before 34 weeks' gestation is in accordance with the results of previous studies on the predictive value of the angiotensin II sensitivity test, a level of 10 ng/kg/min used as the threshold dividing normal and abnormal angiotensin sensitivity.[12] The results of our trial indicate that daily ingestion of 60 mg of aspirin from 28 to 34 weeks' gestation significantly reduces the vascular responsiveness to angiotensin II in angiotensin-sensitive primigravid women. In 82% (95% confidence interval, 57% to 95%) of 17 treated women

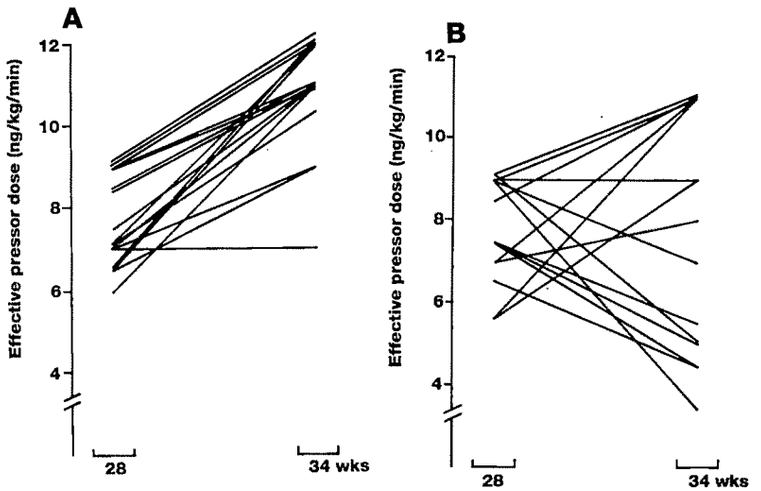


Fig. 1. Minimum amount of angiotensin II (nanograms per kilogram per minute) infused intravenously causing a rise in diastolic blood pressure of 20 mm Hg (effective pressor dose). **A,** In 17 primigravid women at 28 weeks' gestation before and at 34 weeks after 6 weeks of daily oral ingestion of 60 mg of aspirin. **B,** In 15 primigravid women at 28 weeks' gestation before and at 34 weeks after 6 weeks of daily oral ingestion of matched placebo.

but in only 33% (95% confidence interval, 12% to 62%) of 15 women in the placebo group, the effective pressor dose returned to values >10 ng/kg/min. The difference may in fact be more pronounced because of the exclusion of the three women in the placebo group who had become hypertensive at 34 weeks' gestation and, for that reason, most likely had enhanced angiotensin II sensitivity at that time. The fall in heart rate during the effective pressure dose of angiotensin II has been observed by other investigators and may be explained by baroreflex function.[14] Since the fall in heart rate that occurred when the effective pressor dose was reached was equal in both groups, baroreceptor response was apparently not influenced by low doses of aspirin.

Our results are consistent with those of a recent trial in which 22 angiotensin-sensitive women were treated at about 33 weeks' gestation with 81 mg of aspirin during a 1 week period.[14] In that study the mean effective pressor dose increased from 5.9 to 10.2 ng/kg/min, and values of ≥10 ng/kg/min were obtained in 53% of 17 women studied before and after 1 week of low doses of aspirin. However, the study was not placebo controlled or blinded. In an earlier, also uncontrolled study conducted in 13 primigravid women with a physiologic angiotensin refractoriness, the effective pressor dose 2 hours after a single oral dose of 80 mg of aspirin was found to be twice that before treatment.[15] In contrast, oral ingestion of 650 mg of aspirin twice with an interval of 6 hours between doses has been demonstrated to lead to a marked increase in angiotensin sensitivity in pregnant women.[16] The contrasting effect of low and high doses of aspirin may be explained by the finding in various controlled studies in pregnancy[10, 11] and in nonpregnant[17] subjects that long-term administration of aspirin in a daily dose between about 40 and 80 mg markedly reduces the production of platelet thromboxane A_2 while sparing the synthesis of vascular prostacyclin; cumulative higher doses result in progressive inhibition of vascular prostacyclin biosynthesis.[17] The demonstrated selective inhibition of platelet thromboxane A_2 by low doses of aspirin is thought to be due to cumulative presystemic inhibition of cyclooxygenase in platelets passing through gut capillaries with a relatively high concentration of aspirin after oral ingestion, whereas the concentration in the systemic circulation remains too low to affect the enzyme in vascular endothelium.[18] In addition, there are approximately 1000 times as many vascular endothelial cells capable of forming prostacyclin as platelets producing thromboxane A_2.[19] Although we did not measure serum concentrations or urinary excretion of eicosanoid metabolites, the inhibition of the thrombin-induced formation of malondialdehyde by platelets to approximately 10% of the placebo values indicates suppression of thromboxane A_2 dominance and is consistent with the results of other studies in which these metabolites were determined.[10, 11, 14, 20]

Several randomized, placebo-controlled and blinded studies have now confirmed the original report[21] that low doses of aspirin reduce the incidence of pregnancy-induced hypertensive disorders in women with various risk factors,[10, 11, 22] including increased angiotensin sensitivity,[9] most likely because of suppression of platelet thromboxane synthesis and restoration of relative prostacyclin dominance.[10, 11] The finding that a low dose of aspirin improves vascular refractoriness in angiotensin-

sensitive primigravid women supports the hypothesis that enhanced vasopressor sensitivity to angiotensin II is a pathophysiologic mechanism in the development of pregnancy-induced hypertensive disorders associated with an imbalance in prostacyclin–thromboxane A_2 synthesis. The mechanism through which such an imbalance affects vascular reactivity is not fully understood. The vasoconstrictor action of angiotensin II may be enhanced because of an insufficient, direct antagonizing effect of vasodilator prostacyclin. However, the results of a recent study on platelet angiotensin II binding sites[23] suggest that a lack of down regulation of angiotensin II receptors also could be involved.

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Morphologic changes in the human amnion epithelium that accompany labor as seen with scanning and transmission electron microscopy

Jiri Sonek, MD, Steven G. Gabbe, MD, Jay D. Iams, MD, and Douglas A. Kniss, PhD
Columbus, Ohio

Scanning and transmission electron microscopy were used to assess the influence of normal, active labor on the ultrastructure of the human amnion epithelial membrane. Amnion membranes (reflected and placental portions) were obtained from patients either in active labor who were delivered vaginally or by cesarean section after 6 to 12 hours of labor or from patients who underwent elective cesarean section before clinical signs of overt labor. Scanning electron microscopy revealed that reflected amnion membranes that were obtained from patients who were not in labor consisted of a uniform single layer of epithelial cells with numerous microvilli on the apical surface and closely associated cellular borders. In contrast, amnion membranes that were obtained from patients who were in labor consisted of a single layer of epithelial cells, which was interupted by wide intercellular gaps and extracellular extrusions. Transmission electron microscopy showed that intercellular junctions tended to be less complex in patients who were in labor versus patients who were not in labor. Although lipid droplets were prevalent in both patient groups, specimens that were obtained from patients who were in labor had more lipid droplets per cell than specimens from patients who were not in labor. These results support the theory that the complex biochemical events that culminate in parturition are accompanied and/or preceded by demonstrable morphologic changes in the amnion membrane. (Am J Obstet Gynecol 1991;164:1174-80.)

Key words: Parturition, ultrastructure, amnion epithelium, electron microscopy

The microanatomy of the human fetal membranes has been studied previously by a number of investigators.[1-5] It has been noted that the amnion epithelial cells are heterogeneous with respect to light microscopic appearance; there are both "light and dark" staining cells with hematoxylin and eosin.[6-8] Ultrastructural studies have demonstrated that amniotic cells exhibit varying degrees of intracellular vacuolation and lipid droplet accumulation.[3] The fetal membrane specimens that were examined in these previous studies were controlled for a number of clinical parameters that could affect histologic appearance including chorioamnionitis, meconium, and defects that are attributable to birth trauma. One factor, however, which was not taken into account was the influence that active labor had on the structure of the amnion epithelium.

Thus the present investigation was undertaken to examine whether the process of parturition is accompanied by demonstrable morphologic changes in the human amnion epithelium. Reflected amnion and amnion that overlies the placenta from patients at term were examined with scanning and transmission electron microscopy. Amnion membranes were also obtained from patients who were delivered by elective cesarean section without overt signs of active labor. Amnion membranes from patients who were in spontaneous labor were obtained at the time of vaginal delivery in which active labor was 6 to 12 hours in duration.

From the Department of Obstetrics and Gynecology (Division of Maternal-Fetal Medicine), The Ohio State University College of Medicine.
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Reprint requests: Douglas A. Kniss, Department of Obstetrics and Gynecology (Laboratory of Maternal-Fetal Medicine), The Ohio State University, College of Medicine, Columbus, OH 43210.
6/1/26707

Patients and methods

Tissue. Placentas were procured from patients after term delivery (38 to 41 weeks' gestation). Placentas and fetal membranes are excluded under category 5 of the National Institutes of Health guidelines for use of tissues from human subjects. Thus written consent for their collection was not obtained. Confidentiality of patients' identities was protected at all times. Patients with meconium staining, oligohydramnios, hydramnios, or

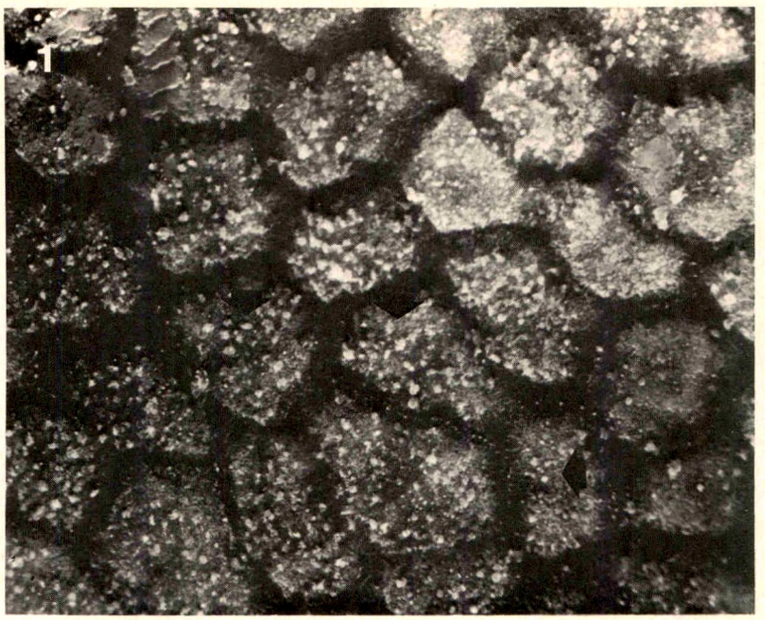


Fig. 1. Scanning electron micrograph of reflected portion of amnion epithelium from a patient who was not in labor which shows a unicellular sheet of closely apposed cells with numerous microvilli that project from the apical surface. *Arrowheads* denote epithelial cells. (Original magnification ×3120.)

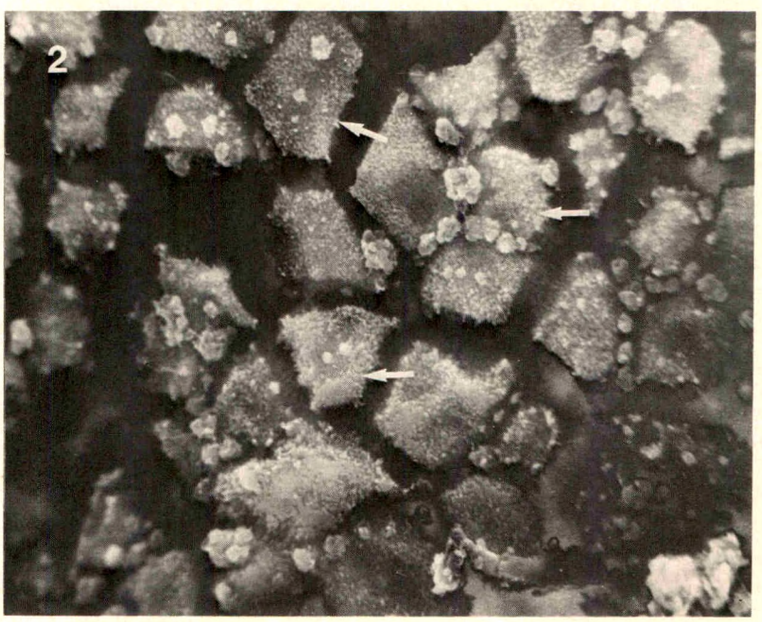


Fig. 2. Scanning electron micrograph of reflected portion of amnion epithelium from a patient who was in labor, which shows separation of intact membrane and deposition of extracellular extrusions. *Arrows* denote epithelial cells. (Original magnification ×3120.)

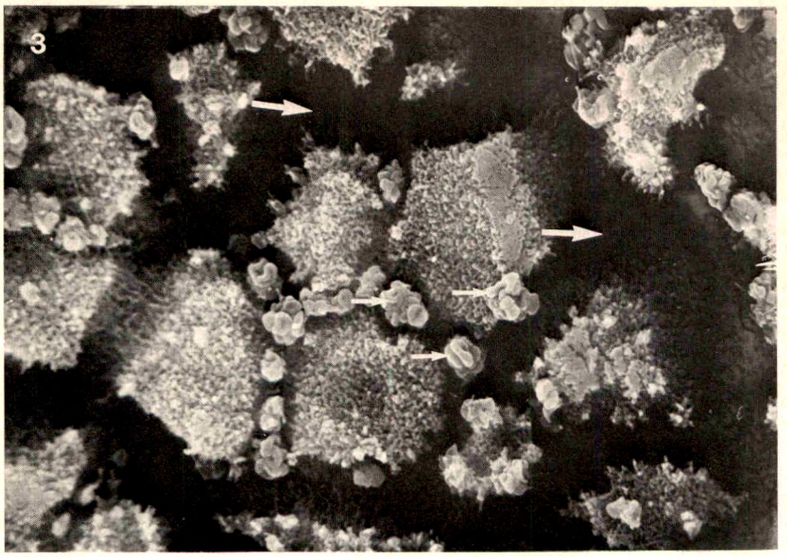


Fig. 3. Scanning electron micrograph of reflected portion of amnion epithelium from a patient who was in labor. *Large arrows* denote wide intercellular spaces. *Small arrows* denote extracellular extrusions. (Original magnification ×7200.)

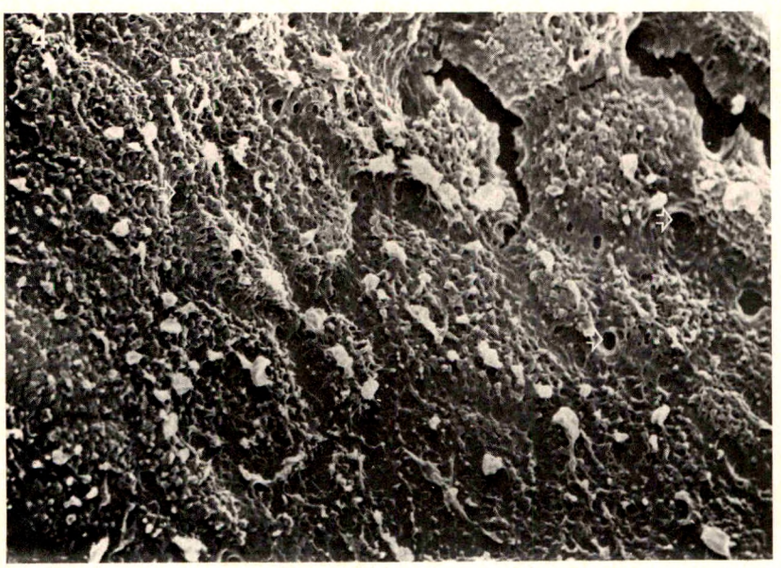


Fig. 4. Scanning electron micrograph of placental portion of amnion epithelium from a patient who was in labor. *Open arrowheads* show ostia to intercellular channels. (Original magnification ×5120.)

suspected chorioamnionitis were excluded from the study. There were no identifiable maternal or fetal risk factors and the patients had not received any medications, including tocolytic agents. Three placentas were obtained from patients with no history of contractions who were delivered by elective cesarean section. One of these patients was primigravid and underwent elective cesarean section for fetal malpresentation, whereas the other two patients were multigravid and had elective repeat cesarean sections. Three placentas were obtained from patients who presented in active labor. The time interval from presentation until delivery ranged from 6 to 12 hours. One primigravid patient received oxytocic augmentation of labor and subsequently underwent a cesarean section for cephalopelvic disproportion after approximately 12 hours of labor. The remaining two multigravid women had normal spontaneous vaginal deliveries after 6 to 12 hours of labor.

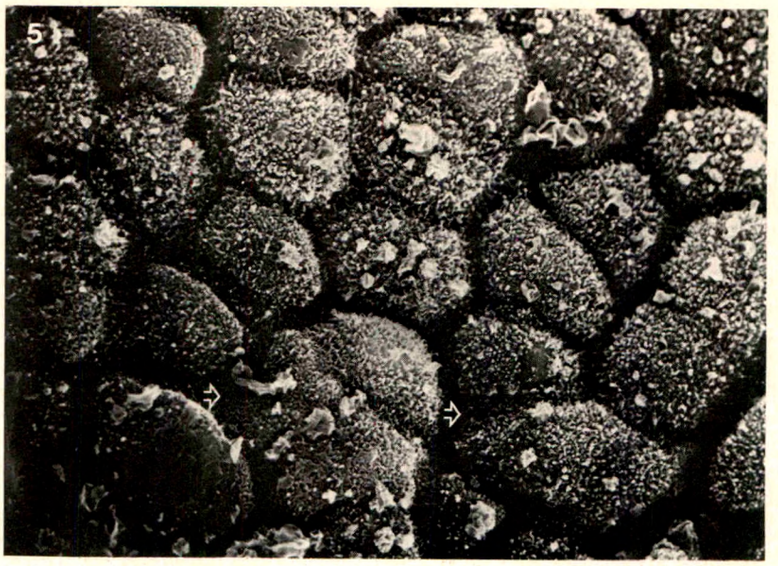


Fig. 5. Scanning electron micrograph of placental portion of amnion epithelium from a patient who was in labor. *Open arrowheads* show wide intercellular spaces. (Original magnification ×3120.)

The placentas and membranes were carefully inspected to rule out any grossly demonstrable abnormalities. All tissues were processed less than 30 minutes after delivery.

Tissue processing. The amnion reflectum was stripped from the underlying chorion, and the placental amnion was stripped from the placental surface. Great care was taken to limit handling of the tissues to minimize trauma-related artifacts. The amnion was then rinsed with sterile phosphate-buffered saline, cut into small pieces (approximately 5 mm^2) and placed in a solution that contained 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer with 5% sucrose for 60 to 75 minutes. The specimens were again rinsed and serially dehydrated in acetone of increasing concentrations from 50% to 100%. Each sample was then divided into two parts, one for preparation for transmission electron microscopy and the other for scanning electron microscopy. In preparation for transmission electron microscopy, the tissue was embedded overnight in Spurr's resin at 60° C. Thick sections (0.5 μm) were cut with glass knives and stained with 1% toluidine blue in borate buffer for orientation. Thin sections (80 to 100 nm) were cut with glass knives and transferred to copper mesh grids. Sections were stained with lead citrate and uranyl acetate and examined with a Philips 300 transmission electron microscope (Philips Electronic Instruments Inc., Mahwah, N.J.) at 60 kV.

In preparation for scanning electron microscopy, the specimens were freeze-dried in a critical point dryer (Pelco 1200, Redding, Calif.) and were then sputtered-coated with gold and platinum. The specimens were examined with a Philips CM-12 scanning-transmission electron microscope in the scanning mode at 40 kV. Amnion reflectum and placental amnion from all six patients were successfully examined by transmission electron microscopy and scanning electron microscopy. At least two preparations from each site were examined with both methods.

Results

Scanning electron microscopy findings

Amnion reflectum. In the tissues that were obtained from patients who were not in labor, the amnion epithelium consisted of a sheet of closely apposed cells with numerous microvilli that projected from the apical surface (Fig. 1). The apical (adluminal) surface of the amnion in patients who were not in labor was more uniform and devoid of cellular extrusions. In specimens that were obtained from patients who were in labor, the intercellular junctions appeared to be widely separated (Figs. 2 and 3; large arrows) and numerous extracellular extrusions (Figs. 2 and 3, small arrows) were noted. The appearance of microvilli was largely unchanged.

Placental amnion. The amnion epithelium from patients who were not in labor consisted of a sheet of cells with poorly defined cellular borders (Fig. 4). The cells appeared to have a raised centrally located area with extracellular material that overlaid it. Infrequently, ostia to intercellular channels were observed (Fig. 4; open arrowheads). The structures of the specimens from patients who were in labor were very different from those in patients who were not in labor. The definition of the cellular borders was more pronounced, and in many places, there were wide intercellular gaps (Figs. 5 and

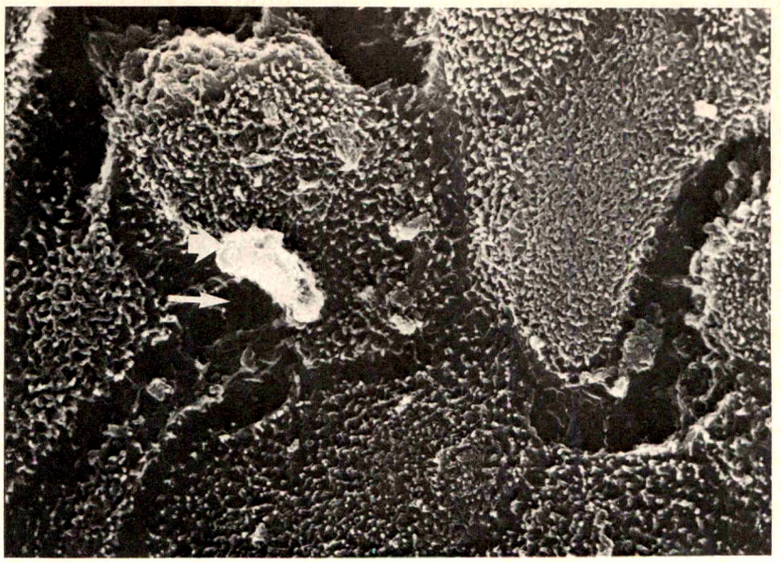


Fig. 6. Scanning electron micrograph of placental portion of amnion epithelium from a patient who was in labor. *Arrowhead* shows extracellular material on surface of epithelial cell. *Arrow* shows opening to intercellular channel. (Original magnification ×7360.)

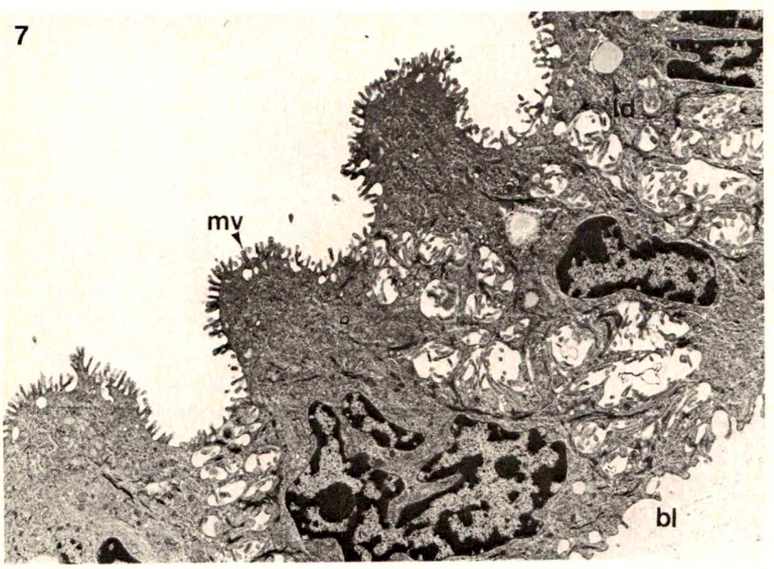


Fig. 7. Transmission electron micrograph of reflected portion of amnion epithelium from a patient who was not in labor. *mv*, Microvilli; *ld*, lipid droplets; *bl*, basal lamina. (Original magnification ×45,900.)

6; open arrowheads). Ostia to the intercellular channels were more numerous in the placental amnion (Figs. 5 and 6; arrows). The extracellular material that overlaid the surface of the cells was largely replaced by clumps of amorphous material of smaller size (Fig. 6; large arrowheads).

Transmission electron microscopy findings

Amnion reflectum. The reflected amnion epithelium in both the patients who were in labor (Fig. 7) and those who were not in labor (Fig. 8) consisted of cuboidal to low columnar cells with centrally located nuclei. The amnion epithelial cells in specimens from patients who

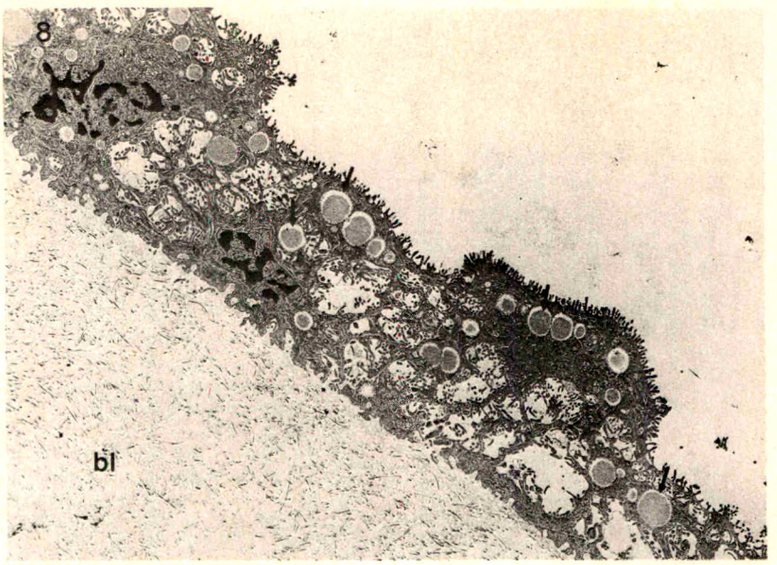


Fig. 8. Transmission electron micrograph of reflected portion of amnion epithelium from a patient in labor. *Arrows* indicate lipid droplets. *bl*, Basal lamina. (Original magnification ×45,900.)

were not in labor were columnar with numerous microvilli that projected from the apical surface (Fig. 7), whereas specimens that were obtained from patients who were in labor had cuboidal epithelial cells. The intercellular junctions in specimens from patients who were in labor tended to be looser and to assume more of a labyrinthine pattern than in those from patients who were not in labor. Similarly, the cellular processes at the base of the cells where they attached to the underlying basal lamina were longer and more complex in the amniotic specimens from patients who were not in labor.

Lipid droplets appeared to be more numerous in the amniotic membranes from patients who were in labor, although they were both present in both groups of specimens (Fig. 8). Further morphometric analysis will be required to ascertain whether these differences are statistically significant. There did not appear to be a consistent difference in the appearance of the nucleus and other cytoplasmic organelles in specimens from patients who were in labor versus specimens from patients who were not in labor.

Comment

The present investigation was undertaken to determine whether the process of normal, active labor influences the ultrastructural features of the human amnion epithelium. Although others have studied the structure of the amnion at term, to our knowledge, this is the first study to be reported in the literature in which the influence of labor was assessed. The human fetal membranes (amnion and chorion laeve) are believed to play a vital role in the maintenance of intrauterine integrity during gestation. The amnion is also known to liberate arachidonic acid and synthesize prostaglandin E_2, which is involved in the initiation of parturition.[9, 10] Our present findings further indicate that these biochemical events are accompanied by or preceded by demonstrable ultrastructural changes in both placental and reflected portions of the amnion membrane.

As a control for the influence of labor on amnion ultrastructure, specimens were obtained from several patients who were delivered by elective cesarean section without overt signs of labor. In these preparations, with the use scanning electron microscopy, the amnion was seen as an intact cellular membrane. In contrast, in patients who presented with overt clinical signs of active labor, the cells of the amnion membrane appeared to be less tightly associated, and large intercellular extrusions between the cells were noted. The nature of these extrusions is not known at the present time. Another intriguing observation was the presence of large amounts of lipid droplets in specimens that were obtained from patients in active labor as compared with patients who were not in labor. Although it must be noted that lipid droplet accumulation was seen in both patients who were in labor and patients who were not in labor, demonstrably more lipid was found in specimens from patients who were not in labor.

In summary, our findings of clearly demonstrable changes in the ultrastructure of the human amnion membrane as a function of active labor lend further

support for an important role of this tissue in the parturitional process. Whether these morphologic changes are the result of the complex biochemical events that take place during labor or are simply ancillary events must await future studies.

We thank Kathleen Wolken and Karen Zaruba for their excellent technical assistance with the electron microscopy. The assistance of Michael Waxman in preparing the electron micrographs and the manuscript is gratefully acknowledged. This work was completed while Dr. Sonek was a fellow in Maternal-Fetal Medicine at The Ohio State University.

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Fibrocystic disease of the breast in premenopausal women: Histohormonal correlation and response to luteinizing hormone releasing hormone analog treatment

Joseph Monsonego, MD,[a] Marie-Dominique Destable, MD,[b] Georges De Saint Florent, MD,[b] Jacques Amouroux, MD,[c] Jean-Claude Kouyoumdjian, MD,[d] France Haour, PhD,[e] Jean-Luc Breau, MD,[a] Lucien Israel, MD,[a] Ana-Maria Comaru-Schally, MD,[f] and Andrew V. Schally, PhD, MD[f]

Bobigny, Creteil, and Paris, France, and New Orleans, Louisiana

Sixty-six patients with fibrocystic mastopathy were enrolled in the trial after being selected according to clinical, radioultrasonographic, and histologic criteria. No characteristic hormonal profile was noted in most patients (52%). Estrogen receptors or progesterone receptors, or both, were found in 57% of patients. Hormone receptor levels were correlated with atypical proliferative mastopathy (87.5%). Mastopathy was associated with a uterine fibroma or a fibromatous uterus in 73% of cases. All patients received intramuscular injections of a sustained delivery system (microcapsules) of luteinizing hormone releasing hormone agonist [D-Trp^6]-LHRH, Ipsen-Biotech, Paris) for 3 to 6 months. In case of partial response at 3 months, an antiestrogen (tamoxifen, 40 mg/day, for estrogen receptor–predominant lesions) or a progestin (cyproterone acetate, 50 mg/day, for progesterone receptor–predominant lesions) was added to the luteinizing hormone releasing hormone agonist. A complete response was observed in more than half of the patients (n = 35, 53%) treated by [D-Trp^6]-LHRH alone (n = 29) or associated with tamoxifen (n = 4) or cyproterone acetate (n = 2). A significant partial response was observed in 30 other patients (45%). Additionally, half of them received inhibitory drugs. The best responses were seen with cyst reformation (complete response, 100%) and fibrous block. Clinical responses to treatment with [D-Trp^6]-LHRH alone were independent of hormone receptor status, but synergistic effects occurred with concomitant use of the corresponding inhibitory drugs. We conclude that chronic mastopathy, particularly when associated with uterine fibroma, can be successfully treated by luteinizing hormone releasing hormone analogs in premenopausal women. (Am J Obstet Gynecol 1991;164:1181-9.)

Key words: Proliferative mastopathy, estradiol receptors, luteinizing hormone releasing hormone analog

The risk of breast cancer is increased in patients with fibrocystic mastopathy when it is associated with epithelial proliferation or family history of cancer.[1] Fibrocystic disease has been interpreted as the result of gonadal or hypothalamic-hypophysial hormonal disorders,[2] but hormonal anomalies[3] or hormonal receptors[4] are not consistently found. Results of various hormonal treatments, including progestins, are incomplete and controversial.[5, 6]

From the Oncological and Breast Screening Unit, Oncology Department, Centre Hospitalier Universitaire, Bobigny,[a] the Thoracic Surgery[b] and Anatomopathology[c] Departments, Hospital Avicenne, Bobigny, the Biochemistry Department, Hospital Henri Mondor, Creteil,[d] the Endocrine Pharmacology Department, Institut Pasteur, Paris,[e] and the Endocrine, Polypeptide and Cancer Institute, Veterans Administration Medical Center and Tulane University School of Medicine, New Orleans.[f]
Received for publication June 18, 1990; revised December 11, 1990; accepted December 21, 1990.
Reprint requests: J. Monsonego, MD, 174 rue de Courcelles, 75017 Paris, France.
6/1/27573

In view of our experience regarding the frequent association of high-risk benign mastopathy with uterine fibromas and the possible effects of luteinizing hormone releasing hormone (LHRH) analogs on both these diseases[7] and in consideration of the reported direct effect of these analogs on breast carcinoma cells,[8, 9] we decided to evaluate the efficacy of [D-Trp^6]-LHRH in chronic fibrocystic mastopathy in premenopausal women with a high risk of breast carcinoma.

Material and methods

Patients. Sixty-six female patients with a mean age of 43 years (range, 31 to 50) participated in this preliminary study, after selection on the basis of criteria of high-risk benign mastopathy.[1] All patients had a regular menstrual cycle of 28 to 30 days and were not using any other drugs.

Fifty patients were premenopausal (mean age, 43.4 years), eight were approaching menopause (mean age, 50.2 years), and eight had undergone conservative total

Table I. Histologic characteristics of mastopathy (according to the Dupont-Page classification)

	No.	%
Nonproliferative disease	10	15
Proliferative disease	56	85
Without atypical hyperplasia	46	70
Typical lobular hyperplasia, including sclerotic adenosis	24 4	36.3
Typical ductal hyperplasia	14	21.2
Pure stromal hyperplasia	5	7.5
Lobular and ductal hyperplasia	3	4.5
With atypical hyperplasia	10	15
Atypical lobular hyperplasia	1	1.5
Atypical ductal hyperplasia (type II-III)	7	10.6
Epitheliosis-papillomatosis (type IV)	2	3

hysterectomy (mean age, 44.7 years). Painful symptoms (58.9%), congestion (60.9%), or hyperesthesia of the nipple (10.15%) was noted in 62 patients (94%). Symptoms was marked in 34 patients (51%), moderate in 22 cases (34%), and mild in 8 (12%). Mastodynia was cyclic in more than three quarters of the cases and continuous in the other 12 cases (18%).

Forty-eight patients (72%) had a family history of cancer, including breast cancer (n = 30) or cancer of the colon (n = 16). A family history of both breast cancer and cancer of the colon was noted in eight cases.

Eight patients (12%) had a history of breast cancer. Twenty-four patients (37%) had undergone breast biopsy and 10 (15%) were obese.

More than half the patients (34/58) were resistant to progestins, and aggravation of mastopathy was noted in 16 patients during treatment. Therapy with tamoxifen alone (30 mg/day) was ineffective in 10 of 30 cases.

Mastopathy. Clinical and radiologic (ultrasonography and mammography) characteristics of mastopathy without regard to histologic findings were noted: macrocysts only (10 cases, 15%); nodules only (16 cases, 25%); fibrous block only (4 cases, 6%); heterogeneous (macrofibronodular) consisting of nodules, cysts, and blocks of various sizes (30 cases, 45%); microcysts only (2 cases, 3%); and clinically normal breasts (4 cases, 6%). This last class of patients was included because of the presence of mastodynia, multipore nipple discharge, radiologically dense breasts, and proliferative mastopathy. Histologic characteristics of mastopathy (according to the Dupont-Page classification[1]) are shown in Table I. Proliferative mastopathy was noted in 56 of 66 cases (85%), and cellular atypia was seen in 10 of the cases (15%), including atypical lobular hyperplasia (n = 1), atypical ductal hyperplasia (n = 7), and epitheliosis (n = 2).

Clinical correlation of histologic findings. Benign mastopathy was heterogeneous. Macrocystic mastopathy (n = 10) was associated with proliferative lesions in four cases (40%), including two with cellular atypia, and nodular mastopathy (n = 16) in 14 cases (80%), including two with cellular atypia. Mastopathy with fibrous block (n = 4) and microcysts (n = 2) was always associated with proliferative lesions, but cellular atypia were absent. Heterogeneous mastopathy (n = 30) was proliferative in 28 patients (94%), including two with cellular atypia. No significant histologic and clinical correlation could be established in this risk group. Proliferative and nonproliferative mastopathies were frequently associated.

Gynecologic examination. Fifty-eight patients (88%) had cyclic menses. The other eight patients had undergone conservative hysterectomy because of fibroma. Fibroma was or had been present in 48 patients (73%), and the uterus was fibromatous in 22 cases (33%). Interstitial or subserous myoma was seen in 16 patients (24%), and one case of submucous myoma was confirmed hysterographically. Polycystic ovaries were seen in six patients (9%) and ovarian endometriosis confirmed by laparoscopy in two patients. Hormonal analysis of plasma prolactin, estradiol, and progesterone was carried out on the twenty-first day of the cycle by double-antibody radioimmunoassay. Three populations were identified: (1) 20 patients (30%) with absolute or relative hyperestrogenism (progesterone/estradiol ratio in nanograms per milliliter, <0.8); (2) 12 patients (18%) with overt hyperprolactinemia (prolactin level, >20 ng/ml) and a normal sella turcica, as assessed by computerized tomographic scan; (3) 34 patients (52%) without hormonal disorders.

Steroid receptor analysis. Analysis of breast biopsy specimens removed surgically on days 20 to 23 of the cycle was used to confirm the presence or absence of hormone receptors (estrogen, progesterone).

Estrogen and progesterone receptors were measured by dextran-charcoal assay. Briefly, fat and connective tissue were removed from tumor specimens, which were then snap frozen and stored at −70° C until assay. Frozen tissue was pulverized, and the powder was thawed and homogenized in assay buffer (0.02 mol/L Tris hydrochloride, 0.04 mol/L sodium molybdate, 10% glycerol, 0.4 mol/L potassium chloride, 0.0015 mol/L ethylenediaminetetraacetate, 0.0025 mol/L dithiothreitol, pH 7.4) with a Polytron (Bioblock Scientific, 67403 Illkirch, France) homogenizer. After centrifugation at 100,000 g for 60 minutes, aliquots of the supernatant were incubated with 7 nmol/L [^{3}H]17β-estradiol or 20 nmol/L ^{3}H-ORG 2058 (Amersham) in the presence of either a 100-fold excess of unlabeled diethylstilbestrol or assay buffer. After a 16-hour incubation at 4° C, bound and free labeled hormone was separated with dextran charcoal. Cytosol binding ca-

pacity, calculated from the difference between bound and free radioactivity, correlated significantly with the Scatchard analysis ($r = 0.97$, $n = 45$ for estrogen receptors; $r = 0.96$, $n = 16$ for progesterone receptors) of the results of assays performed in parallel with various concentrations of labeled hormone. All results were expressed per milligram of cytosol protein, as determined by the method of Lowry et al. Levels of estrogen and progesterone receptors >10 fmol/mg cytosol protein were defined as significant. Receptor assay was not possible in six patients. Estrogen-receptor protein was present (estrogen receptor positive; mean, 84 fmol/mg protein) in 26 cases (44%) and progesterone-receptor protein was present (progesterone receptor positive; mean, 112 fmol/mg protein) in 24 cases (40%). Eighteen patients (30%) were both estrogen and progesterone receptor positive, and 28 patients (43%) were both estrogen and progesterone receptor negative. This is probably due to tissue heterogeneity of the lesions and is in agreement with previous findings.[4, 10] In contrast to the case of breast adenoma,[11] no clear correlation was noted here between receptor content and estradiol and progesterone levels during the menstrual cycle. This likely reflects probably unregulated genomic control of receptor levels at the lesion boundary, as seen in breast cancer.[12] There was no significant correlation between the clinical type of mastopathy and the hormone receptor content. There was, however, a clear correlation between epithelial proliferation with cellular atypia and hormone receptor content. Of 10 cases of atypical proliferative mastopathy, eight of which were assayed for hormone receptors, seven (87.5%) showed elevated levels and six were classified as estrogen receptor positive. Six of 10 cases (60%) of non-proliferative mastopathy were classified as hormone receptor negative. Four of five (80%) cases of pure stromal hyperplasia were classified as hormone receptor negative. These results corroborate literature reports that hormone receptor levels, estrogen receptor levels in particular, are correlated with the degree of epithelial hyperplasia.[4, 10, 11]

LHRH receptors analysis. LHRH receptors on biopsy specimens can be visualized and measured by quantitative autoradiography.[13] Frozen slices are exposed to a radioactive tracer ([D-Ala^6]-LHRH ethylamide labeled with iodine 125; specific activity, 2000 Ci/mmol). After incubation and washing, the slices are placed over radiosensitive films (Amersham ^{3}H Hyperfilm, Amersham, 91944 Les Ulis, France). After exposure, the pictures are quantified by picture analysis (Biocom, Rag 2000). This technique of direct visualization does not show specific (high-affinity) receptors in the biopsy specimens of mastopathy; the appearance of nonspecific receptors is significant in the vascular coating (low affinity and high capacity) in particular, and the marker does not correlate with the presence of specific receptor sites.

Treatment. Informed consent was obtained from all patients before treatment with the long-acting LHRH analog [D-Trp^6]-LHRH (Ipsen-Biotech, Paris) administered every 4 weeks by intramuscular injection of 3.75 mg in the form of sustained-release microcapsules releasing 100 μg analog per day for 28 days. The first injection was given in the first 5 days of the menstrual cycle. After 3 months, treatment was modified according to the response: For a complete response, treatment was discontinued; for a partial response, in all cases treatment with [D-Trp^6]-LHRH was continued for three more months in combination with tamoxifen 40 mg/day for patients with estrogen receptor–predominant lesions or cyproterone acetate 150 mg/day in cases of progesterone receptor–predominant lesions.

These therapies were chosen for their estrogen-antagonist property in benign mastopathy.[5-11] Tamoxifen counteracts the proliferative effect of estrogens by competing with them at the estradiol receptor level. Cyproterone acetate is a progestin, and therefore one of its effects is to yield a decrease in estradiol receptor concentration. This antiandrogen is well tolerated. It has no negative metabolic effect such as norsteroid progestins have.

Moreover, the hypothalamic-pituitary-ovarian axis blockade by [D-Trp^6]-LHRH suppresses the consequences of the clomiphene-like effect of tamoxifen and the metrorrhagia induced by cyproterone acetate before the menopause.

Clinical and radiologic evaluation of mastopathy. The size and number of cysts, nodules, or blocks evaluated during examination at the third and sixth months were compared with the results of the first examination. The response was considered complete when these structures were no longer present at 3 or 6 months. The response was considered partial when the mean diameter of mastopathy structures had decreased after treatment. These decreases ranged from 20% to 80%. Any effects of the concomitant use of tamoxifen and cyproterone acetate were evaluated and scored as synergistic, nil, or antagonistic. A second clinical and gynecologic evaluation was performed after the six treatment cycles. This was supplemented by comparative mammography, mammary and pelvic ultrasonography, and hormonal analysis that was identical to that performed at the start of the trial.

Results

Mastopathy

Mastodynia. Pain was absent in all cases ($n = 62$, 100%) after 2 months of treatment. Exacerbation of mastodynia was seen in 18 cases (28%) between day 0

Table II. Response rates of components of mastopathy to LHRH analog, alone and in combination with tamoxifen and cyproterone acetate

Component of mastopathy	*Complete response*		*Partial response*		
	3 mo	*6 mo*	*A**	*B†*	*C‡*
Nodules (n = 42)		6 (15%)	28§ (66%)	8 (19%)	
Block (n = 34)		12 (35%)		19‖ (56%)	3 (9%)
Cyst formation (n = 12)	12 (100%)				
Nipple discharge (n = 18)		12 (67%)		6 (33%)	

*80% decrease in number or mean diameter of mastopathy structures.
†60% decrease in number or mean diameter of mastopathy structures.
‡40% decrease in number or mean diameter of mastopathy structures.
§With the addition of tamoxifen, n = 12; with the addition of cyproterone acetate, n = 7.
‖With the addition of cyproterone acetate, n = 2.

and day 15. This corresponded to the first stage of hormonal stimulation. These symptoms subsided around day 20 in all cases.

Clinical and radiologic (ultrasonography-mammography) responses according to type of mastopathy. Stabilization and regression (complete response) were seen after 3 months in all cases of macrocystic mastopathy (n = 10) previously treated by cyst aspiration. Pure fibronodular mastopathy (n = 16) had subsided completely at 6 months in 25% of cases (4/16). Nodules decreased in number and volume at 6 months in 50% of cases (8/16). A favorable response was induced in the remaining patients (25%) by concomitant use of tamoxifen (2 cases) or cyproterone acetate (2 cases) with [D-Trp^6]-LHRH from the third month to the sixth month (when the receptor status was estrogen receptor positive or progesterone receptor positive, respectively). In mastopathy involving fibrous block (n = 4), the volume of affected tissue had decreased by less than half in two cases. Concomitant use of cyproterone acetate during the last 3 months of treatment resulted in a 60% regression in fibrous block in two other patients (progesterone receptor positive). Heterogeneous (n = 30) or macrofibronodular mastopathy had subsided completely at 6 months in 16 patients, six of whom had received either tamoxifen (n = 4, estrogen receptor positive) or cyproterone acetate (n = 2, progesterone receptor positive) as a companion drug during the last 3 months of treatment. The volume of affected tissue decreased approximately twofold in one patient after 3 months. Overall, the most frequent mastopathy disappeared in 54% of cases after 6 months and subsided more than twofold in the other 46%. Microcystic mastopathy (2 cases) regressed completely at 3 months (complete response, 100%).

After 6 months of treatment with [D-Trp^6]-LHRH alone, multiple surgical biopsy specimens were done to evaluate atypical proliferative mastopathy in four patients with clinically normal breasts. No epithelial hyperplasia was noted in any of the biopsy specimens taken from three of the four patients. Cellular atypia was still present in one patient.

Response in terms of symptoms is recorded in Table II. Nodules (n = 42) regressed completely at 6 months in six patients (15%) and had decreased in size and number in 17 patients receiving [D-Trp^6]-LHRH alone, in 12 women treated with the combination of [D-Trp^6]-LHRH and tamoxifen (estrogen receptor), and in seven patients receiving concomitant cyproterone acetate (progesterone receptor positive). Regression (complete response) of fibrous block (n = 34) was noted in 12 patients (35%) at 6 months. The volume of fibrous block was reduced to one half in the remaining 19 patients (56%) receiving [D-Trp^6]-LHRH alone (n = 20) or in combination with cyproterone acetate (progesterone receptor positive, n = 2). When cysts (n = 12) were aspirated, recurrence was not seen (complete response, 100%) after 3 months of treatment. Multipore nipple discharge without cytologic anomalies (n = 18) resolved after 6 months of treatment in 12 patients (67%) and decreased in the other 6 (33%). Hypermastia diminished by 30% in 10 of 14 patients (70%).

In 45 patients receiving [D-Trp^6]-LHRH alone, complete responses were noted in 29 (65%) at 3 (n = 12) or 6 months (n = 17). Partial responses were recorded in 15 patients (33%) at 3 (n = 1) and 6 months (n = 14). One patient with proliferative mastopathy without clinical failure had no response after histologic control. Responses were not complete after 3 months of treatment with [D-Trp^6]-LHRH in 21 patients, 19 of whom were classified as hormone receptor positive. At 6 months, after concomitant use of cyproterone acetate (n = 9) or tamoxifen (n = 12), complete regression was noted in 6 of these patients (28%), 80% regression in 10 (48%), and 60% regression in five (24%). Resolution of all types of mastopathy was noted in 35 cases (53%),

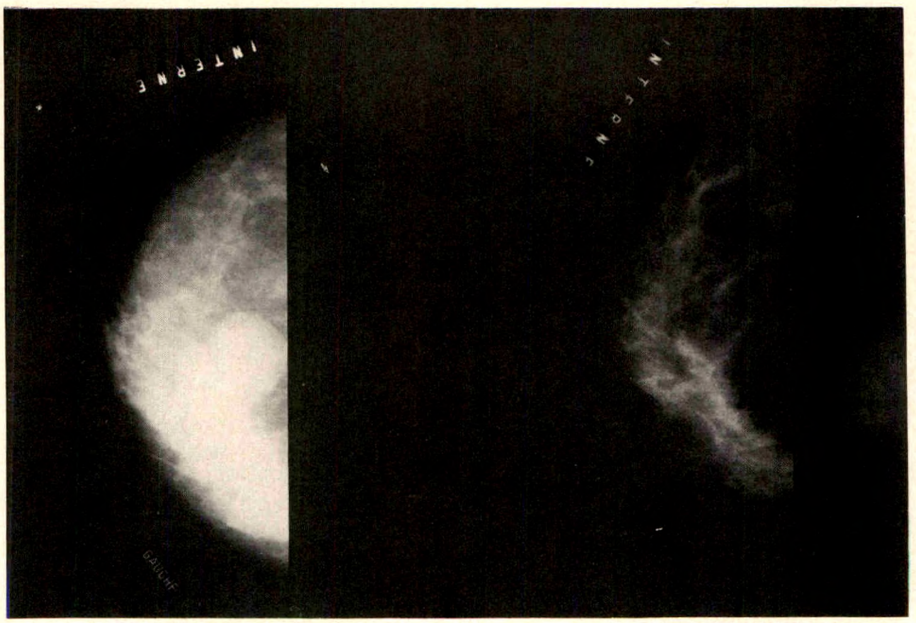


Fig. 1. Mammography before *(left)* and after *(right)* treatment by [D-Trp^6]-LHRH. We noted the cyst (aspirated before treatment) and more opacity, which regressed after 6 months of treatment.

Table III. Clinical, ultrasonographic and radiologic responses of all types of mastopathy to LHRH analog ([D-Trp^6]-LHRH) alone and in combination with tamoxifen and cyproterone acetate

	Complete response		*Partial response*			
Mastopathy	*3 mo*	*6 mo*	*A**	*B†*	*C‡*	*No response*
Mastopathy with clinical features (n = 62)§	12 (19%)	20 (32%)‖	22 (36%)¶	5 (8%)#	3 (5%)	—
Proliferative mastopathy without clinical features** (n = 4)		3				1
TOTAL		35 (53%)		30 (45%)		1

*80% decrease in number or mean diameter of mastopathy structures.
†60% decrease in number or mean diameter of mastopathy structures.
‡40% decrease in number or mean diameter of mastopathy structures.
§One patient had a partial response at 3 months and stopped the treatment.
‖With the addition of tamoxifen, n = 4; with the addition of cyproterone acetate, n = 2.
¶With the addition of tamoxifen, n = 8; with the addition of cyproterone acetate, n = 2.
#With the addition of cyproterone acetate, n = 5.
**A histologic control has been made before and after treatment.

12 at 3 months and 23 at 6 months. More than twofold regression in mastopathy was noted in other cases (n = 30), at 3 (n = 1) or 6 (n = 29) months (Table III). Mammography at 6 months indicated a clear decrease in glandular density in the majority of cases (Fig. 1).

Clinical, ultrasonographic, and mammographic responses according to hormonal receptor status. The responses of nodules, cysts, and fibrous block to treatment were independent of hormone receptor status, but synergistic response was noted with concomitant use of antisteroidal agents (tamoxifen and cyproterone acetate). Complete remission was most frequent (71.5%) in the estrogen and progesterone receptor–negative group, which was characterized by the absence of cellular atypia and which was treated with [D-Trp^6]-LHRH alone. Hormone-dependent lesions (estrogen and

Table IV. Clinical and radiologic responses according to hormonal receptor status*

	Complete response		*Partial response*		
	[D-Trp⁶]-LHRH alone	*[D-Trp⁶]-LHRH plus tamoxifen or cyproterone acetate*	*[D-Trp⁶]-LHRH alone*	*[D-Trp⁶]-LHRH plus tamoxifen or cyproterone acetate*	*Total*
Hormone receptor negative†	20 (71.5%)		8 (28.5%)		28 (46%)
Hormone receptor positive‡	9 (28%)	4 (12.5%)	4 (12.5%)	15 (47%)	32 (54%)

*Receptor assays were not possible in six patients.

†Hormone receptor negative: estrogen and progesterone receptor negative.

‡Hormone receptor positive: estrogen receptor positive and progesterone receptor negative, estrogen receptor negative and progesterone receptor positive, estrogen and progesterone receptor positive.

progesterone receptor positive, estrogen receptor negative and progesterone receptor positive, estrogen receptor positive and progesterone receptor negative) responded to treatment with [D-Trp⁶]-LHRH alone (9/32) and to combination therapy with [D-Trp⁶]-LHRH with tamoxifen or with cyproterone acetate during the last 3 months after a partial response (15/32). In the case of tamoxifen, synergism was particularly correlated with estrogen receptor–positive cases, which in this study were most often associated with cellular atypia (Tables IV and V).

Associated gynecologic anomalies. Mastopathy was associated with a fibromatous uterus or uterine myoma in 48 patients. In 26 cases of fibromatous uterus a complete remission was noted at 3 and 6 months with a return to normal size in eight and six cases, respectively; i.e., complete response was achieved in 54% of cases. Uterine volume decreased by >50% (partial response) in eight other patients. No response was noted in four obese patients. No response to treatment was noted in two cases of submucous myoma. Partial responses (>50%) were seen in 18 of 20 patients with subserous and interstitial myoma. Overall, for all types of fibroma, complete responses were seen in 30% of cases at 3 or 6 months. More than twofold decreases in size were noted for the uterus and myomas in 54% of cases at 6 months. No response was found in eight (16%) patients, six of whom were obese. Complete remission of cysts was noted at 3 months in all six cases of polycystic ovary.

Laparoscopy indicated that when ovarian endometriosis was associated with mastopathy, it responded to treatment in two cases after 6 months.

Long-term follow-up. All patients were seen regularly, twice a year. After a mean of 2½ years of follow-up, we have been able to assess mammary and gynecologic relapse. Menstruation returned after cessation of treatment in all patients who had had regular cycles: 75 days after the last injection in 40 patients and 3 months later in 18 patients. No mammary relapse was noted, but some nodules and block recurred. Among the patients with complete response, the lesions reappeared (nodules, 2/6; block, 5/12; cysts, 6/12) but less frequently than in the group of patients with partial response (nodules, 15/36; block, 11/22). It is noteworthy that during this period no aggravation of the mastopathy was observed. Therefore, if the lesions recur, their number and size always remain smaller than before treatment. Among the patients with clinical symptoms, complete response remained without relapse in 50% of cases (15/32). This long-term response rate is also observed in the group of patients with partial response (15/30).

This suggests that [D-Trp⁶]-LHRH has a lasting effect in some cases. Follow-up therapy could be considered in other patients.

Uterine fibromas recurred sooner, and the increase in size was estimated to be 65% (31 cases) for all types of fibroma after a mean of 2½ years of follow-up.

Side effects. Hot flushes were reported by 64 patients (97%) and increased with time of treatment. Only 30% of these patients responded to additional nonhormonal treatment. Asthenia developed with time, was always present at 6 months, and responded best in 90% of cases to adjuvant vitamin B treatment. Forty of the 66 patients (60%) reported cephalalgia, which decreased with time and responded to additional therapy. Other manifestations (depression, vaginal mucous atrophy, decreased libido, visual disorders, hypertension) were relatively rare (4.6% of cases) and well tolerated. A flareup effect was seen in 18 patients (27%) 10 to 15 days after the first injection. This was due to drug-induced stimulation of the pituitary gonadal axis at the start of treatment and was marked by recurrence of mastodynia or onset of metrorrhagia. These manifestations were mild and generally subsided before the second injection of the analog. Providing that a partial response had been achieved with [D-Trp⁶]-LHRH (10/12), synergism was seen when tamoxifen was used as an adjunctive drug. No estrogenic effect of tamoxifen was seen. In two cases the inhibitory effect of

Table V. Clinical and radiologic responses according to estradiol receptor status*

	Complete response		*Partial response*		
	[D-Trp⁶]-LHRH alone	*[D-Trp⁶]-LHRH plus tamoxifen or cyproterone acetate*	*[D-Trp⁶]-LHRH alone*	*[D-Trp⁶]-LHRH plus tamoxifen or cyproterone acetate*	*Total*
Estrogen receptor negative	22 (65%)	0	8 (23.3%)	4 (11.7%)	34 (51.6%)
Estrogen receptor positive	7 (27%)	4 (15.4%)	4 (15.4%)	11 (43%)	26 (44%)

*Receptor assays were not possible in six patients.

[D-Trp⁶]-LHRH was nullified by the clomiphene-like stimulatory effect of tamoxifen. Metrorrhagia, leukorrhagia, and ovarian cysts developed in these two cases, indicating that the suppressing effect of [D-Trp⁶]-LHRH had been counteracted by peripheral estrogen-like activity induced by tamoxifen. Combination therapy with [D-Trp⁶]-LHRH and cyproterone acetate was well tolerated, and synergism was seen in all cases (9/9). The gynecologic side effects (metrorrhagia) of cyproterone acetate were counteracted by the estrogen-depriving activity of [D-Trp⁶]-LHRH.

Comment

This is the first report of the use of an LHRH analog in the treatment of chronic mastopathy. In our study true or relative hyperestrogenism before treatment was noted in 30% of patients and overt hyperprolactinemia was present in 18%. Hormone receptors were present in 54% of patients, and 44% of patients were classified as estrogen receptor positive.

Although LHRH analogs are effective in most cases of severe mastopathy, their mechanism of action is incompletely understood. LHRH analogs act mainly by creating a state of estrogen deprivation, but it has also been suggested that they block ovarian steroidogenesis.[14]

Estrogens,[15] progesterone,[6] androgens,[16] and prolactin[17] influence cellular proliferation in the mammary gland in vitro. This action is mediated by the corresponding receptors, all of which are found in potentially malignant tissue. It has been shown in this study, and by other investigators, that in proliferative mastopathy estrogen receptors are correlated with the degree of epithelial hyperplasia[10] and cellular atypia and that progesterone receptors are correlated with the extent of stromal proliferation. The potent antigonadotropic action of LHRH agonists induces complete ovarian inhibition, which results in very low blood levels of estradiol, progesterone, ovarian androgens, and prolactin, in turn as a result of decreased blood estradiol. This indirect effect of high doses of LHRH analogs has been reported in premenopausal metastatic breast cancer, in which chemical castration gives significant results in 30% to 40% of cases.[8] In the present study, however, complete response to [D-Trp⁶]-LHRH alone was noted in only 27% of cases of estrogen receptor–positive mastopathy, 25% of which were atypical, indicating that the decrease in blood estradiol was not adequate to control the process and reverse the course of the disease.

In the normal breast estrogen receptors are present only at low levels in the first phase of the cycle.[12] In this study, with most patients not having luteal impairment, the presence of hormone receptors at day 20 of the menstrual cycle may indicate a possible disturbance of genomic regulation of estrogen receptors, the production of which may be independent of the hormonal cycle. This has been reported in breast cancer[18] but with more elevated levels of estrogen receptor than in proliferative mastopathy. It is also possible that local estrogens produced from androgens sustain to some extent the trophic function of the mammary gland (estrogen receptor), as has been suggested for the normal breast in menopausal women.[12]

In the second 3-month treatment period, synergistic action of the inhibitory agents tamoxifen and cyproterone acetate was related to the receptors found and was noted in 60% of hormone receptor–positive patients showing complete response (4/32) or partial (>60%) response (15/32). The synergism noted with tamoxifen was correlated mainly with estrogen receptor–positive cases, in which cellular atypia was often present. Other authors[19] have related a synergistic effect of the two drugs in estrogen receptor–negative breast carcinoma. Strong blocking of the ovary and pituitary ensured good drug safety and helped eliminate the clomiphene-like effects of tamoxifen and cyproterone acetate–induced metrorrhagia. Ovarian hyperstimulation was seen in only one of 12 patients receiving [D-Trp⁶]-LHRH and tamoxifen concomitantly.

Although hypophysial antagonism of the two drugs has been described,[8] it is rare with high doses of long-acting LHRH analogs. Although the hormonal mechanisms clearly exist, there are nonetheless some cases of mastopathy in which no endocrinologic disorders are

observed[3] and no increase in hormonal receptor content is found. In this study more than half the patients had normal hormone levels and were classified as estrogen receptor negative.

The best results with [D-Trp^6]-LHRH alone were seen in estrogen receptor–negative, progesterone receptor–negative patients (complete response in 71% of cases), characterized by glandular hyperplasia and the absence of cellular atypia. However, immunohistochemical determination showed that receptors could be present in some cases in this group.[10] The mechanism of action of LHRH analogs in hormone-independent groups is unknown. A strong effect in this subgroup of patients suggests that the drug may act directly. Direct in vitro action of LHRH analogs has been reported in breast cancer lines.[20] They inhibit the growth of MCF-7 cells, particularly when primed by estradiol. High-affinity LHRH receptors have been identified in various human mammary cancer lines[21] and in 260 of 500 biopsy specimens of human breast cancer.[22] However, receptors identified in some extrahypophysial tissues (testis, placenta) generally seem to display low affinity for LHRH agonists. The antiproliferative action of LHRH analogs on mouse mammary gland cells may be greater than that of ovariectomy. A direct antiproliferative action is supported in a study of the effects of in vivo administration of LHRH analog to mice on subsequent in vitro proliferation of MXT mammary tumor cell cultures (containing estrogen and progesterone receptors).[23] LHRH analogs are effective in postmenopausal women with breast cancer in whom blood estrogen levels are not affected (10% to 25% of cases). LHRH agonists also show some activity in premenopausal and postmenopausal estrogen receptor–negative breast cancers.[8, 24]

We used a quantitative autoradiographic analysis to study 20 cases of proliferative mastopathy. This technique permitted us to localize LHRH receptors in brain and in human ovaries.[13] We found that the receptors were present in 12 cases, but these binding sites were mainly of low affinity. Various explanations have been advanced to resolve the discrepancy between the marked in vitro and in vivo antiproliferative effects of LHRH agonists and their low affinity for receptors. It is possible that high concentrations of LHRH analogs have a biologic effect on weak cellular response, as seen in the placenta. The mitogenic action of the pituitary gonadotropins follicle-stimulating hormone and luteinizing hormone[23] is suppressed in the presence of LHRH analogs. However, this has not been verified in human breast cancers. We have noted a frequent association among proliferative mastosis, family history of breast cancer, and uterine fibroma. The risk for breast cancer seems to be increased by this clinical association in these diseases, which is sensitive to treatment with LHRH analogs.[7] The risk of malignancy linked to the first two factors and the beneficial effects of LHRH analogs on fibroma are already well known.[25] Our demonstration of the efficacy of these analogs in the treatment of high-risk benign mastopathy should help in the assessment of the therapeutic efficacy of this drug. As in the case of mastopathy, uterine fibromas are influenced by estrogen levels and have LHRH receptors.[26] However, mastopathy does not always involve increases in estrogen receptors. Antiestrogenic agents do not give such consistent results with major mastosis and fibromas as those that can be obtained with LHRH analogs, alone or in combination therapy. Tamoxifen was ineffective in one third of the cases in our study. Proliferation of mesenchymal cells (smooth muscle of the uterus, breast myoepithelium) is common in high-risk mastopathy and uterine fibroma. This was particularly evident in mastopathy with block, which was resolved by treatment with [D-Trp^6]-LHRH in 50% of cases in this study. There is an important fibromatous component in all cases of mastopathy. Its role in breast oncogenesis has been reported.[7] A harmonious interaction between the stroma and epithelial cells is required for tissue homeostasis. Myoepithelial cells play an important role in the development of breast cancer, as indicated by their special role in the basal membrane, their lack of estrogen receptors[10] and LHRH receptors, and their ability to secrete various endogenous growth factors.

In hormone-independent mastopathy, LHRH agonists may act at the stroma, which generally does not contain hormone receptors. However, it is not known if the initial trigger for cellular proliferation is associated with stromal proliferation or is induced by hormone-sensitive glandular cells. Fibrous proliferation does not reflect cicatrization but does indicate the presence of an active tumor, which regresses when the inducing stimulus is removed.[16] The role of fibrosis in oncogenesis has been demonstrated in many tissues, and interactions between stromal and glandular cells have been reported.[27] In mastopathy, LHRH analogs seem to control mesenchymal proliferation and thus could affect tumoral growth factors. Further studies are needed to confirm this and to define the effect of LHRH analogs on the epithelium and stroma.

In conclusion, high-risk fibrocystic mastopathy, particularly when associated with uterine fibroma, can be successfully treated by LHRH analogs in premenopausal women. Although these analogs improve clinical and radiologic manifestations of mastopathy, it remains to be seen whether they control epithelial proliferation at the subclinical stage. It is also necessary to determine the short- and long-term effects of estrogen deficiency. A short treatment (4 to 6 months) can control the progress of the disease without serious effects on bone density and gives better long-term results with mastopathy

than with fibroma. Larger scale within-subject studies are necessary to confirm the high risk present in this population (proliferative mastopathy, family history of cancer or uterine fibroma) and to establish whether breast cancer prevention by treatment with LHRH analogs is possible in these cases.

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Fast-scan magnetic resonance imaging in fetal visualization

Anne S. Garden, MB, ChB,[a] Richard D. Griffiths, MD,[b] A. Michael Weindling, MD,[c] and Peter A. Martin, PhD[d]

Liverpool, United Kingdom

Magnetic resonance imaging has potential as an imaging technique in obstetrics. Its application has been limited by the amount of image degradation that occurs as a result of fetal movement during the long imaging times. By use of a fast-scan imaging technique good images are obtained of fetal anatomy in 3 to 14 seconds per image, depending on the setting used. Different organs could be visualized by varying the setting. Magnetic resonance imaging complements the role of ultrasonography in fetal visualization. It also allows clear fetal imaging in such high-risk situations as maternal obesity and oligohydramnios, where it may be difficult to produce clear images by ultrasonography. This technique will allow wider application of magnetic resonance imaging in obstetrics and give additional information about the developing fetus. (Am J Obstet Gynecol 1991;164:1190-6.)

Key words: Magnetic resonance imaging, fast-scan technique, fetal visualization, fetal movement, fetal anatomy

Accurate fetal imaging is an essential part of feto-maternal medicine. Obstetric imaging techniques should be safe, noninvasive, and capable of providing good tissue definition in both normal and abnormal pregnancies.

Magnetic resonance imaging (MRI) fulfills these criteria. Its application to obstetrics has, however, been limited because of long imaging times. Conventional spin-echo nuclear magnetic resonance requires imaging times of 5 to 45 minutes[1] to produce a series of images. As each image is built up throughout this time, fetal movement causes distortion of the whole series. Fetal movement can be seen on ultrasonographic ex-

From the Departments of Obstetrics and Gynecology,[a] Medicine,[b] and Child Health[c] and the Magnetic Resonance Imaging Centre,[d] University of Liverpool.
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Reprint requests: Miss A.S. Garden, Senior Lecturer, Department of Obstetrics and Gynaecology, Royal Liverpool Hospital, Prescot St., Liverpool, United Kingdom L7 8XP.
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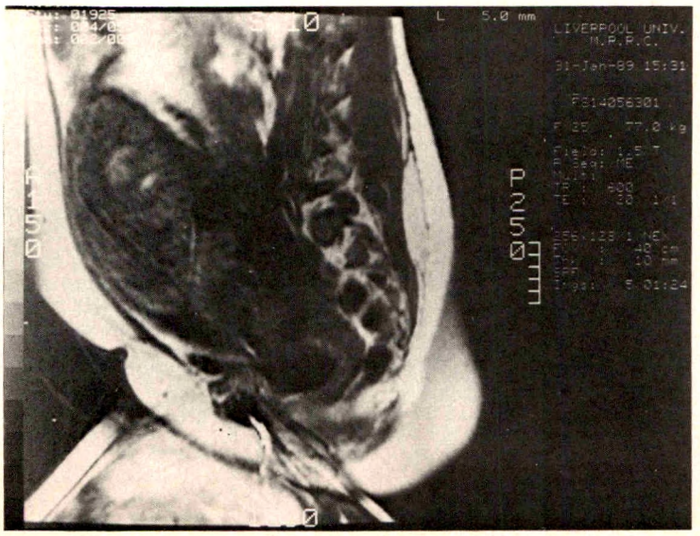


Fig. 1. Conventional spin-echo magnetic resonance imaging (repetition time, 600 msec; echo delay time, 20 msec) with complete loss of fetal visualization because of motion of nuclei.

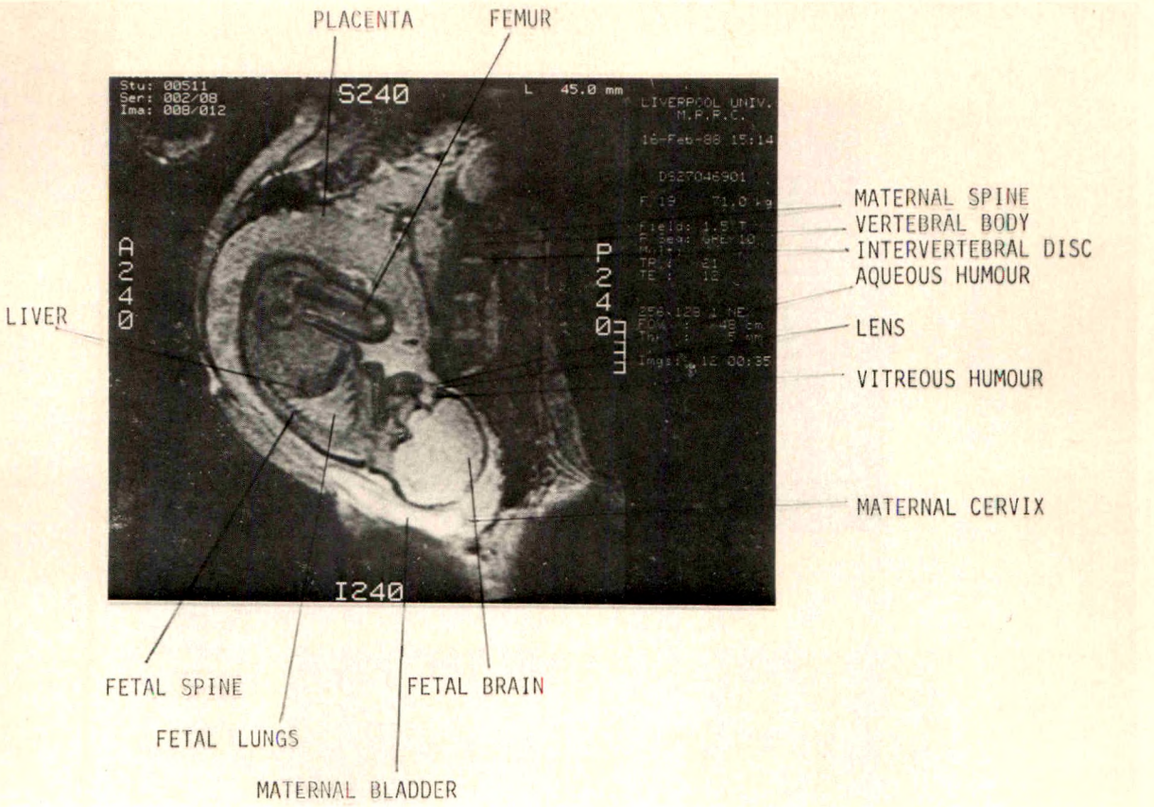


Fig. 2. Repetition time, 25 msec; echo delay time, 12 msec; flip angle, 10 degrees. Fetal anatomy clearly seen, including fetal lung, liver, brain, bone, and subcutaneous tissues.

amination from 7 weeks' gestation and becomes more frequent and complex with advancing gestation. Even at term gross body movements occur. Such fetal movements cause image degradation with MRI. Further distortion also occurs because of the movement of amniotic fluid nuclei. The combination of these two phenomena may cause such a degree of distortion as to give the appearance of an empty uterus. Almost all researchers in the field quote fetal movement as the main problem to be overcome and have limited its application in the study of growth and development of fetal organs. Administering benzodiazepine to the mother[2] or curare to the fetus[3] has been suggested as a means of reducing fetal movement. This is unacceptable for routine use, particularly with a technique that is otherwise noninvasive.

We describe a new application of a fast-scan imaging technique[4, 5] that is aimed at extending the use of MRI in obstetrics and facilitating the study of the developing fetus.

Conventional spin-echo MRI involves the application of a 90-degree radio-frequency pulse to the magnetic field. Time must be allowed between each excitation for the magnetic field to recover. This interval, the repetition time, is generally between 500 and 2000 msec. For the acquisition of reasonable images, the radio-frequency pulse must be repeated many times, and so a long repetition time results in relatively long imaging times. In fast-scan MRI a radio-frequency pulse that causes only a small excursion in the magnetization is applied. In this way the length of time taken to obtain an image can be shortened to a few seconds. Clearer fetal images are produced because of reduced movement artifact but at the expense of reduced signal strength. An additional advantage is that movement occurring during imaging causes distortion of only that single image in the series.

Patients and methods

A General Electric Signa system was used. Imaging was performed at 1.5 T with the standard body coil within the guidelines laid down by the National Radiological Protection Board in 1983.[6] Ethical permission was obtained from the local and regional scientific committees.

Sixty-seven women were recruited for the study from the antenatal clinics and wards. Twelve pregnancies were studied on two occasions. The studies were carried

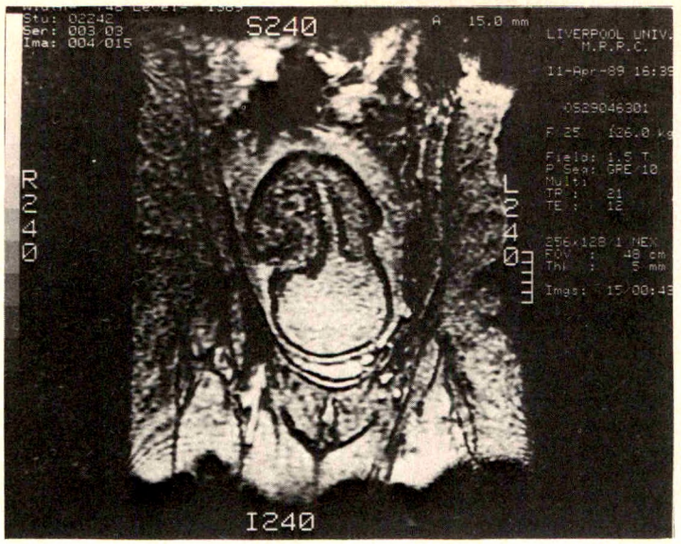


Fig. 3. Visualization of fetal brain and cervical spine in obese mother weighing 126 kg.

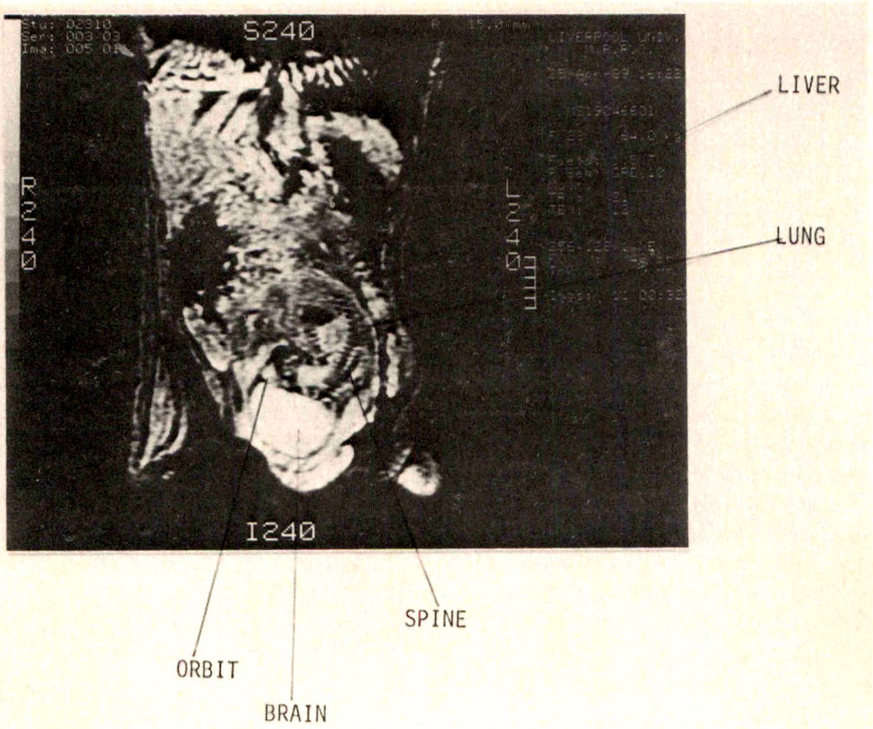


Fig. 4. Premature rupture of membranes at 30 weeks' gestation. Fetal anatomy including brain, vertebrae, lungs, and orbit seen despite lack of amniotic fluid.

out between 28 and 40 weeks' gestation. Forty-two of the women studied had uncomplicated pregnancies, and the remaining 25 gestations were complicated. Complications included suspected congenital anomalies (eight patients), polyhydramnios (three patients), medical disorders complicating the pregnancy (three patients—insulin-dependent diabetes in two and hemochromatosis in one), placenta previa (two patients),

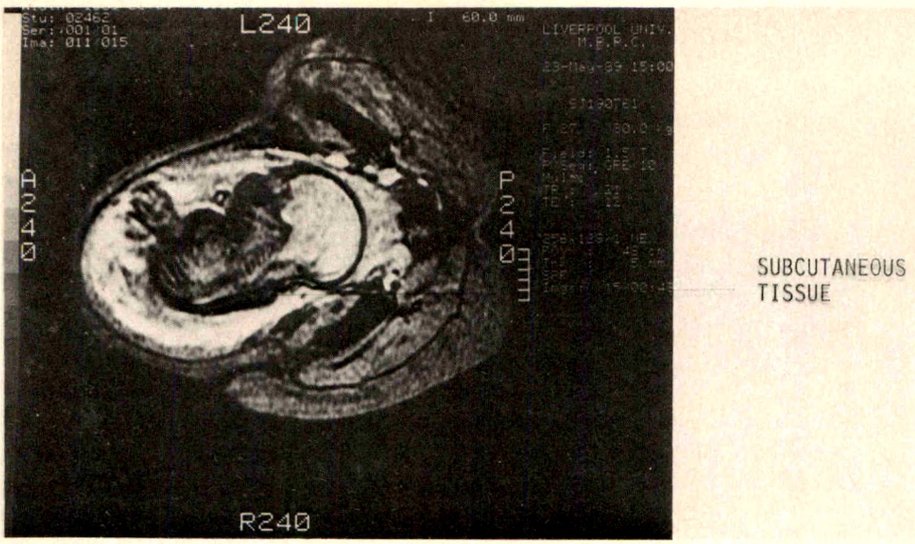


Fig. 5. Increased thickness of subcutaneous tissue in macrosomic baby of insulin-dependent diabetic mother.

suspected intrauterine growth retardation (two patients), prolonged premature rupture of the membranes with subsequent oligohydramnios (three patients), suspected macrosomia (two patients), and twin pregnancy complicated by intrauterine death of one twin (two patients).

With the fast-scan technique, imaging was carried out with the woman lying in the right lateral position. Foam padding was used to support the pregnant uterus and to maintain the woman's position comfortably within the magnet bore.

Initial fetal localization was carried out in the maternal axial, sagittal, and coronal planes. From these, fetal alignment could be ascertained and further imaging carried out in planes that gave optimum fetal visualization. Images were obtained at varying echo delay time, repetition time, and flip angles to assess the best settings for visualizing the fetal anatomy.

Results

No serious adverse effects were encountered. Three patients felt slightly claustrophobic on first entering the magnet bore but were able to continue with the study after assurance. Only one study had to be abandoned because of this.

With the conventional spin-echo settings, it was not possible to consistently and reliably image the fetus. On most occasions the appearance of an empty uterus was obtained (Fig. 1).

The best views of the fetus were considered to be those in which fetal organs could be easily identified. With a short repetition time of 25 msec, a short echo delay time of 12 msec, and a flip angle of 10 degrees, the fetal heart, liver, lungs, and bone were clearly seen (Fig. 2) and a clear outline was obtained of the fetal skin and subcutaneous tissue. With this setting, good visualization could be obtained in all clinical situations, including maternal obesity (Fig. 3) and oligohydramnios (Fig. 4). It also allowed measurement of the fetal subcutaneous tissue as an indicator of macrosomia (Fig. 5). A single image with this setting could be obtained in 3 seconds.

Similar echo delay time (12 msec) and repetition time (25 msec) but with a larger flip angle of 45 degrees, showed the anatomy less clearly but the fluid in the fetal bowel and umbilical cord was more distinct (Fig. 6). Clearer visualization of fluid in the fetal heart, vessels, and bowel (Fig. 7) was obtained with a repetition time of 100 msec, echo delay time of 12 msec, and flip angle of 60 degrees. This setting, however, required 14 seconds to acquire a single image that consequently increased image distortion because of movement.

A repetition time of 100 msec, echo delay time of 30 msec, and a flip angle of 30 degrees produced strong signals from the brain (Fig. 8). A single slice was obtained in 13 seconds.

The peak specific absorption rate for radiofrequency exposure in these studies ranged from 0.017 W/kg for the faster settings to 0.105 W/kg for the slower settings required for the studies of the intracranial anatomy.

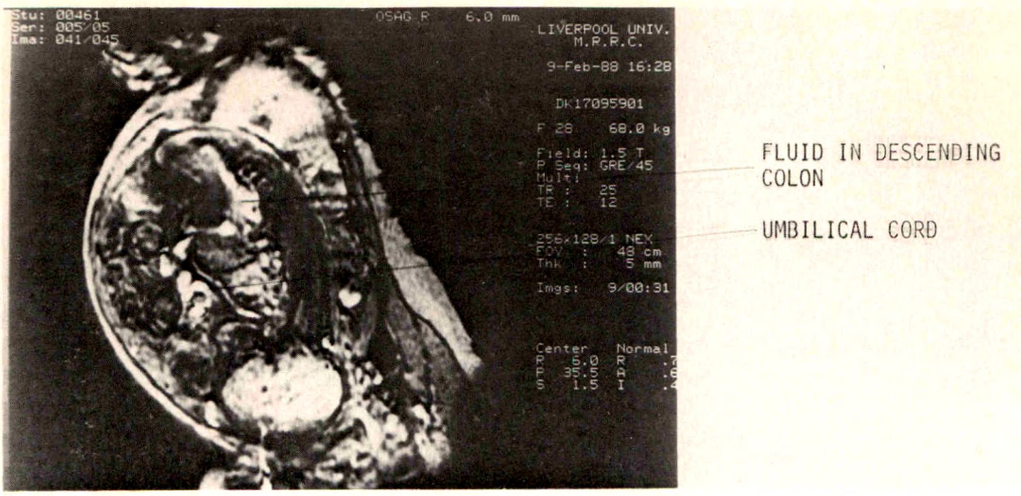


Fig. 6. Repetition time, 25 msec; echo delay time, 12 msec; flip angle, 45 degrees. Fetal anatomy less distinct than in Fig. 1, but fluid in descending colon and umbilical cord clearly visible.

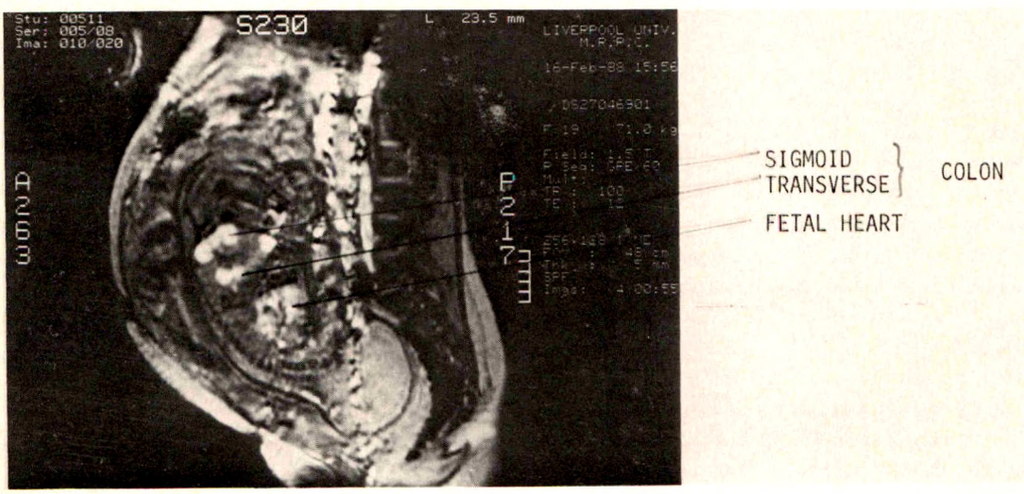


Fig. 7. Repetition time, 100 msec; echo delay time, 12 msec; flip angle, 60 degrees. Fetal anatomy less distinct than in Fig. 2, but fluid in bowel and fetal heart visible. Artifact caused by flow in maternal vessel.

Comment

The safety of MRI has been considered in many studies. Biomedical investigation by means of bacteria or cell culture, in vivo studies, and clinical follow-up of patients has failed to identify any adverse effects.[7-10] Three specific areas have been considered by the National Radiological Protection Board—the effects of the static magnetic field; the time-varying magnetic field effect, which may cause the induction of electric currents; and heat production by the radio-frequency magnetic fields. The guidelines laid down by the board are aimed at preventing these potential hazards.[6] McRobbie and Foster[11] in a study of pregnant mice showed no significant difference between the litter numbers and growth rates of the exposed litters compared with controls. However, the National Radiological Protection Board has advised against imaging during the first trimester, i.e., the period of organogenesis, unless the patient is undergoing termination of pregnancy.

The peak specific absorption rates obtained in this study of 0.017 to 0.105 W/kg are well within the limits

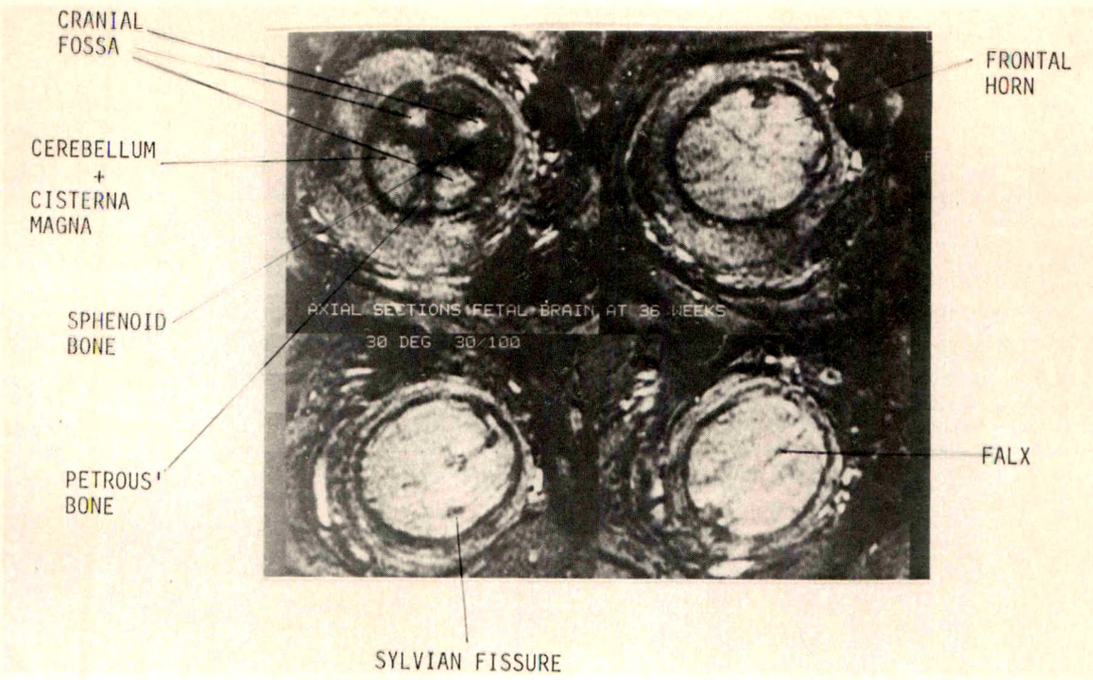


Fig. 8. Repetition time, 100 msec; echo delay time, 30 msec; flip angle, 30 degrees. Detail of intracranial anatomy seen.

laid down by the National Radiological Protection Board[6] and compare favorably with the spin-echo study peak specific absorption rates of 0.577 W/kg.

Other studies with the conventional spin-echo technique have shown that MRI has potential in obstetric imaging.[12, 13] Long imaging times, however, have limited its application. The fast-scan technique described here overcame that problem.

Clearer images are obtained by reduction in motion artifact, and, in addition, the short imaging time allowed image alignment so that fetal anatomy could be visualized by design rather than fortuitously. Other workers in the field have commented on the difficulty of doing this with the conventional spin-echo technique.[14] Using the fast-scan technique, we have been able to image, in every patient studied, the fetal brain, spinal cord, skeleton, lungs, heart, liver, limbs, subcutaneous tissue, and placenta. Details of intracranial anatomy and kidneys have been less consistently visualized. Clear images were also obtained in patients with oligohydramnios or maternal obesity. Ultrasonographic images obtained in these situations give poor image definition.

A further benefit was that the maternal position could be maintained without difficulty within the magnet bore. This has been found to be a problem with the long imaging times required for conventional scanning.[15]

MRI, in contrast to ultrasonography, cannot be used at the bedside nor does it give real-time images. Echo-planar MRI is being developed[16, 17] and has great potential. The image quality at present is not as good as that obtained by fast-scan MRI, however, and requires equipment that is not currently commercially available. The fast-scan imaging technique described here is suitable for all later generations of scanners currently available and installed in many hospitals.

Therefore MRI does fulfill the criteria for obstetric imaging. It is safe, noninvasive, and can be used in both normal and abnormal pregnancies. Although the expense of the unit and the current lack of a real-time facility prevent its superseding ultrasonography as the first-line obstetric imaging technique, it certainly has a complementary role. In particular, it gives superior soft-tissue images and may provide useful additional information about fetal growth and organ development.

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The N-terminal and C-terminal portions of the atrial natriuretic factor prohormone increase during preeclampsia

Rhene W. Merkouris, MD,[a] Frank C. Miller, MD,[a] Val Catanzarite, MD, PhD,[a] J. Gerald Quirk, Jr., MD, PhD,[a] Lee A. Rigg, MD, PhD,[a] and David L. Vesely, MD, PhD[b]

Little Rock, Arkansas, and Tampa, Florida

The influence of preeclampsia on the circulating concentrations of the 28-amino-acid carboxy terminus (C-terminus) (i.e., atrial natriuretic factor) and the amino terminus (N-terminus) of the 126-amino-acid atrial natriuretic factor prohormone (pro ANF) was studied in the third trimester with the use of three specific radioimmunoassays that recognize: (1) atrial natriuretic factor (i.e., amino acids 99 to 126), (2) the whole 98-amino-acid N-terminus, and (3) amino acids 31 to 67 from the midportion of the N-terminus of the prohormone. The C-terminus was significantly increased ($p < 0.001$) in the third trimester in women with preeclampsia, the mean ± SEM of 15 subjects was 150 ± 7 pg/ml versus 89 ± 7 pg/ml in the third trimester in 12 women during normal pregnancies and 65 ± 2 pg/ml in 19 healthy nonpregnant women. The whole 98-amino-acid N-terminus, likewise, was significantly increased ($p < 0.001$) in women with preeclampsia to 4706 ± 629 pg/ml versus 2160 ± 79 pg/ml in women in the third trimester of normal pregnancies and versus the circulating concentration of 1847 ± 127 pg/ml in healthy nonpregnant women. ProANF 31 to 67 mean circulating concentration in preclampsia was 4638 ± 725 pg/ml, which was also significantly ($p < 0.001$) increased compared with its mean circulating concentration in the third trimester of normal pregnancy of 1758 ± 83 pg/ml or that in healthy nonpregnant women (1400 ± 105 pg/ml). The circulating concentrations of both the N-terminus and C-terminus of the atrial natriuretic factor prohormone decreased within 24 hours after delivery in contrast to a normal pregnancy in which they both increase post partum. These results indicate a marked difference in the metabolism of both the N-terminus and the C-terminus of the atrial natriuretic factor prohormone in women with preeclampsia versus that in women with normal pregnancies or that in healthy nonpregnant women. (AM J OBSTET GYNECOL 1991;164:1197-202.)

Key words: Preeclampsia, atrial natriuretic factor prohormone, proteinuria, hypertension, edema

Atrial natriuretic factor (ANF), the 28-amino-acid carboxy terminus (C-terminus) end of the 126-amino-acid ANF prohormone that is synthesized in cardiac myocytes, has natriuretic, diuretic, and potent vasodilator properties.[1] Recently, it has been found that peptides from the amino terminus (N-terminus) end of this prohormone, that is, amino acids 1 to 30 (pro ANF 1 to 30; long-acting sodium stimulator) and amino acids 31 to 67 (pro ANF 31 to 67; vessel dilator) also have vasodilatory,[2] natriuretic, and diuretic properties that are similar to ANF.[3] The vasodilatory effects of prohormone ANFs 1 to 30 and 31 to 67, as well as those of ANF (i.e., amino acids 99 to 126 of the prohormone) are associated with a fourfold to fivefold increase in cyclic guanosine monophosphate, which is due to activation of particulate guanylate cyclase (EC 4.6.1.2).[2] With the use of specific and sensitive radioimmunoassays the whole N-terminus of the ANF prohormone and a 3900 to 4000 peptide from the midportion of the N-terminus that is consistent with prohormone ANF 31 to 67 have recently been demonstrated to circulate normally in humans.[4-8]

Human ANF is found in increased concentrations in the blood of women with preeclampsia compared with the blood of normal pregnant women.[9, 10] Preeclampsia is not accompanied by the 40% plasma-volume expansion that is seen in a normal pregnancy.[11] Rather, preeclampsia is associated with increased generalized vasoconstriction.[12] This vasoconstriction is believed to cause an increased volume load to the cardiac atria which results in enhanced release of ANF into the circulation.[13] Since the N-terminus of the ANF prohormone is released simultaneously with physiologically induced central hypervolemia (i.e., "head-out-of-water" immersion),[14] the present investigation was de-

From the Department of Obstetrics and Gynecolgy, University of Arkansas for Medical Sciences,[a] and the Departments of Medicine, Physiology, and Biophysics, University of South Florida for Health Sciences, James A. Haley Veterans Hospital.[b]

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Reprint requests: Rhene W. Merkouris, MD, Department of Obstetrics and Gynecology, University of Arkansas for Medical Sciences, 4301 West Markham, Slot 518, Little Rock, AR 72205.

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Table I. Clinical data on 15 women with preeclampsia

Patient No.	*Age (yr)*	*Gestational age*	*Weight gain during pregnancy*	*Blood pressure (mm Hg)*	*Heart rate (beats/min)*	*Proteinuria (mg/dl)*	*Edema*
1	34	31	18	160/110	74	100	−
2	23	36	14	172/120	88	300	+
3	28	38	17	131/98	80	30	+
4	16	40	10	140/110	82	300	+
5	24	31	15	140/100	88	300	+
6	16	37	17	149/98	80	100	+
7	18	36	20	144/100	88	100	+
8	27	28	21	150/110	96	300	+
9	20	25	16	170/120	76	2000	+
10	23	31	9	148/110	84	2000	+
11	22	40	20	160/110	72	100	+
12	24	40	10	152/100	88	100	+
13	16	37	13	150/100	88	100	+
14	23	30	12	180/120	88	2000	+
15	28	29	15	181/106	92	100	+
Mean ± SD	23 ± 5	35 ± 5	14 ± 5	156/108 ± 14/8	85 ± 7	529 ± 741	

signed to compare the circulating concentration of the N-terminus of the prohormone in the circulation of women during pregnancies that are complicated by preeclampsia and again in these same women after delivery. This was done by utilization of sensitive and specific radioimmunoassays that recognize amino acids 1 to 98 and 31 to 67 of the ANF prohormone.

Patients and methods

Patients. Fifteen pregnant women between the ages of 16 and 34 years (mean ± SD = 24 ± 5 years) fulfilled the criteria for preeclampsia of (1) a diastolic blood pressure > 90 mm Hg and (2) excretion of > 1 gm of protein per day.[15] Their blood pressures ranged from 136/98 mm Hg to 180/120 mm Hg with a mean of 156/107 mm Hg; heart rates ranged from 72 to 96 beats/min (mean, 85 ± 7) (Table I). Two of the preeclamptic patients (nos. 2 and 10) had a documented history of hypertension that antedated these pregnancies. They were receiving methyldopa, labetolol, hydralazine, and Aldactazide when they were admitted because of preeclampsia; the other 13 patients were not receiving any medication except iron and vitamin supplements. All women were admitted to the maternity unit of the University Hospital of Arkansas at least 2 days before the study was performed. The preeclamptic women consumed a diet of 120 mEq/day of sodium while they were in the hospital. They all had activity limited to bed rest with bathroom privileges. No medications were given for 24 hours before or during the study. Gestational age was estimated by obstetric history and confirmed by fetal ultrasonography. The amount of proteinuria was evaluated by the dipstick method in which 1+ = 30 mg/dl, 2+ = 100 mg/dl, 3+ = 300 mg/dl, and 4+ = 2000 mg/dl protein. During labor all of the preeclamptic subjects received intravenous magnesium sulfate, and eight subjects (1, 2, 5, 8, 9, 10, 14, 15) were given hydralazine to control blood pressure during labor or delivery or during the first 24 hours after delivery.

All plasma samples that were to be assayed for the C-terminus and the N-terminus of the ANF prohormone were obtained from the antecubital vein while each subject was in a sitting position. Informed consent was obtained from each of the 15 volunteers with preeclampsia after the nature and possible consequences of the study were fully explained. This investigation was approved by the Human Use Committee of the University of Arkansas for Medical Sciences.

Methods. Radioimmunoassays to measure the N-terminus of the prohormone were devised to amino acids 1 to 30 and 31 to 67 of the 126-amino-acid prohormone; the C-terminus assay recognizes amino acids 99 to 126 of the prohormone, that is ANF as previously described by our laboratory.[4-8] Gel-permeation chromatography with G-50 Sephadex revealed that the prohormone ANF 1 to 30 radioimmunoassay recognizes a peptide of 10,000 molecular weight which is consistent with the whole N-terminus (i.e., amino acids 1 to 98 of the prohormone) but lacks the C-terminus (i.e., ANF).[8] Thus although the prohormone ANF 1 to 30 radioimmunoassay was devised to recognize synthetic amino acids 1 to 30 of the 126-amino-acid prohormone, it immunologically recognizes the whole 1 to 98 amino acid segment that makes up the N-terminus of the prohormone. The prohormone ANF 31 to 67 radioimmunoassay, on the other hand, recognizes a peptide of 3900 to 4000 molecular weight from the midportion of the N-terminus of the prohormone (actual ANF prohormone 31 to 67 molecular weight = 3878).[8] Similarly,

Table II. N-terminus (i.e., amino acids 1-98), midportion of N-terminus (i.e., pro ANF 31-67), and C-terminus (ANF) of ANF prohormone in preeclampsia

	Pro ANF 1-98 (pg/ml)		*Pro ANF 31-67 (pg/ml)*		*ANF (pg/ml)*	
Patient No.	*Third trimester**	*Post partum†*	*Third trimester**	*Post partum†*	*Third trimester**	*Post partum†*
1	2532	1556	2669	1701	132	67
2	3008	1697	2938	1668	143	72
3	2318	2070	2842	2023	90	65
4	3508	1731	3428	1714	166	74
5	8963	2976	9585	3135	136	76
6	3364	1865	3009	1778	125	71
7	3532	1921	3366	1889	141	70
8	8139	2263	9921	2715	173	85
9	9643	1884	9758	2348	190	79
10	7665	1919	7280	1981	196	82
11	3576	1656	2148	1342	156	80
12	2452	1219	2237	1521	119	69
13	3910	2178	2740	1964	144	68
14	4066	2239	3836	2774	180	86
15	3908	1806	3811	1793	163	77
Mean ± SEM	4706 ± 629	1933 ± 99	4638 ± 725	2023 ± 126	150 ± 7	75 ± 2
Nonpregnant (n = 19) (mean ± SEM)	1847 ± 127		1400 ± 105		62 ± 5	

*Circulating concentrations of pro ANF 1 to 98, pro ANF 31 to 67, and ANF in women with preeclampsia significantly increased ($p < 0.001$) when compared with the circulating concentration of these peptides in nonpregnant women when evaluated by one-way analysis of variance.

†The circulating concentrations of pro ANF 1 to 98, pro ANF 31 to 67, and ANF decreased significantly ($p < 0.001$) 24 hours after delivery compared with the predelivery preeclamptic concentrations when evaluated by paired Student's t test.

G-50 Sephadex gel permeation chromatography revealed that the ANF radioimmunoassay recognizes a peptide of approximately 3000 molecular weight, and the actual molecular weight of ANF is 3081.

Blood samples were placed in 5 ml EDTA (ethylenediaminetetraacetic acid) tubes and immediately centrifuged at 3000 g for 15 minutes. After centrifugation, the plasma was extracted with 100% ethanol (1:1 dilution), mixed by vortexing, and allowed to stand at 4° C for 30 minutes. After 30 minutes at 4° C, the samples were centrifuged at 3000 g for 15 minutes, and the supernatants were dried via controlled nitrogen flow. The samples were then ready for radioimmunoassay. All determinations were performed in triplicate. The intraassay coefficient of variation for prohormone ANFs 1 to 30, 31 to 67, and ANF radioimmunoassays were 4.8%, 5.3%, and 5.7%, respectively. The interassay coefficient of variation was 8% for both prohormone ANFs 1 to 30 and 31 to 67 radioimmunoassays, whereas the interassay variation of ANF was 6.9%. Recovery was examined by the addition of synthetic unlabeled prohormone ANF 1 to 30, prohormone ANF 31 to 67, and ANF at 100, 200, and 400 pg/ml to pooled plasma. Recovery (mean ± SD) for prohormone ANF 1 to 30 was 83.5% ± 13.2% and for prohormone ANF 31 to 67 recovery was 100.9% ± 8.9%. Recovery of ANF was 92% ± 11%. The respective 50% inhibitory concentrations were 180, 120, and 11 fmol per tube whereas the lowest detectable concentrations were 40, 35, and 1.4 fmol for prohormone ANFs 1 to 30, 31 to 67, and ANF radioimmunoassays, respectively. Serial dilution of pooled plasma has revealed excellent parallelism of the standard and the unknown in these assays.[5, 8]

Statistical analysis. All values are reported as mean ± SEM. Differences between groups were analyzed by one-way analysis of variance. The differences between samples in the same individual were evaluated by Student's t test for paired data.

Results

The circulating concentrations of each of the ANF prohormone peptides were significantly elevated ($p < 0.001$) in blood that was obtained from the pregnant women with preeclampsia compared with concentrations in nonpregnant women (Table II). Specifically, the mean concentration of the whole N-terminus (4706 ± 629 pg/ml) in the 15 women with preeclampsia was two times higher than mean blood concentrations in 19 healthy nonpregnant women who had concentrations of 1847 ± 127 pg/ml.[4] The midportion of the N-terminus, which consists of amino acids 31 to 67 of the ANF prohormone (i.e., prohormone ANF 31 to 67), in women with preeclampsia was three times higher than its mean circulating concentration in 19 nonpregnant women (4638 ± 725 pg/ml vs 1400 ± 105

pg/ml).[4] The C-terminus (i.e., ANF) of the ANF prohormone was two times higher in women with preeclampsia than in healthy nonpregnant women (Table II).

This mean concentration of the N-terminus in the third trimester of pregnancy with preeclampsia was also significantly higher ($p < 0.001$) than its mean circulating concentration of 2160 ± 79 pg/ml in 12 women during the third trimester of normal pregnancy.[16] The mean concentration of prohormone ANF 31 to 67 in the third trimester was also significantly higher ($p < 0.001$) than its circulating concentration of 1758 ± 83 pg/ml in 12 women during the third trimester of normal pregnancy.[16] The mean circulating concentration of the C-terminus was nearly two times higher than its mean circulating concentration of 89 ± 7 pg/ml in 12 women during the third trimester of normal pregnancy.[16]

The circulating concentration of the whole N-terminus, the midportion of the N-terminus, and the C-terminus of the ANF prohormone correlated roughly with the severity of the preeclampsia. The subject with the mildest preeclampsia (No. 3 in Table I) had the lowest circulating concentrations of the N-terminus, prohormone ANF 31 to 67, and the C-terminus, whereas two of the subjects with the most severe preeclampsia (No. 9 and No. 10 in Table I), on the basis of severity of hypertension and amount of proteinuria, had two of the highest circulating concentrations of these peptides. Two other subjects with severe preeclampsia as determined by amount of proteinuria (Nos. 5 and 8 in Table I) also had very high circulating concentrations of the whole N-terminus, prohormone ANF 31 to 67, and ANF. One patient with severe preeclampsia (No. 14), however, did not have as high a circulating concentration of these peptides as did the other subjects with severe preeclampsia, although this subject's (No. 14) circulating concentrations of all three peptides were twice that of women in the third trimester of normal pregnancies. Thus in general, the circulating concentration of all three of these peptides correlated with the severity of the preeclampsia.

After delivery, the levels of the whole N-terminus, the midportion of the N-terminus, and the C-terminus of the ANF prohormone decreased significantly ($p < 0.001$) within 24 hours (Table II). Although the levels of the N-terminus, prohormone ANF 31 to 67, and ANF did not decrease completely into the normal range for all women with preeclampsia during this time period, in every preeclamptic subject there was a marked decrease after delivery. In general, the subjects with the highest circulating concentrations of these peptides were those who did not attain the circulating concentration of a nonpregnant person within 24 hours. It is important to note that in some persons with severe preeclampsia such as subject No. 5, the circulating concentrations of the C-terminus returned to the upper levels of the normal range within 24 hours, whereas the whole N-terminus and the midportion of the N-terminus (i.e., prohormone ANF 31 to 67) were still significantly ($p < 0.05$) elevated.

Comment

Although measured plasma volume is usually reported to be decreased in preeclampsia,[11, 17] there is a generalized increase in vasoconstriction as preeclampsia becomes more severe.[12, 17] This vasoconstriction causes an increased localized volume load to the cardiac atria, which results in enhanced release of ANF into the circulation.[13] In the present investigation, as in previous studies,[9, 10, 13] the level of ANF was found to be markedly increased in preeclampsia compared with the circulating concentration of ANF during a normal pregnancy. In addition to ANF (i.e., the 28-amino-acid C-terminus of the 126-amino-acid ANF prohormone), we found that the N-terminus of this prohormone increased in the plasma of women with preeclampsia compared with its circulating concentration in both normal nonpregnant[4, 8] and pregnant women[16] as determined by two sensitive and specific radioimmunoassays. One of these radioimmunoassays (i.e., prohormone ANF 1 to 30) immunologically recognizes the whole N-terminus of the prohormone (i.e., amino acids 1 to 98 of the prohormone), and the second N-terminal assay (prohormone ANF 31 to 67) recognizes a midportion of the N-terminus (amino acids 31 to 67).[8] Prohormone ANF 31 to 67 circulates in the blood as a distinct peptide with natriuretic properties[3] after it has been cleaved from the rest of the prohormone by proteases.[8] Although this is the first investigation that demonstrates a significant increase in the N-terminal portion of the ANF prohormone during preeclampsia, with the use of two separate assays, it appears that both the N-terminus and the C-terminus increase in the circulation of women with preeclampsia. Central hypervolemia does simultaneously release both the N-terminus and the C-terminus of the ANF prohormone,[14] but whether central hypervolemia is the cause or the result of the marked elevation of both the N-terminus and the C-terminus of the ANF prohormone in preeclampsia is uncertain at present.

If hypervolemia with an increased release of the N-terminus and C-terminus was the whole cause of the markedly increased circulating levels in preeclampsia, one might wonder, why in normal pregnancy with its marked increase in plasma volume, the levels of the N-terminus and the C-terminus of the ANF prohormone were not higher than preeclamptic levels.[16] The exact role of a localized volume load to the heart with in-

creased circulating concentration of the C-terminus and the N-terminus could not be ascertained from the present study.

The contribution of heart rate to the increased circulating levels of prohormone peptides also could not be determined with certainty from the present study. With cardiac pacing, however, the increased release of the N-terminus and the C-terminus of the ANF prohormone is not observed until the rate is 125 beats/min.[7] Since none of the women with preeclampsia had a heart rate above 96 beats/min, one would suspect that heart rate did not contribute significantly to the increased concentrations of the N-terminus and the C-terminus of the ANF prohormone in preeclampsia.

Another possible contributor to the increased concentrations of the N-terminus and C-terminus of the ANF prohormone may be the hypertension that is associated with preeclampsia. Atrial natriuretic factor is known to be increased in certain forms of hypertension such as the high blood pressure that is associated with essential hypertension,[18, 19] with pheochromocytomas,[20] and with the hypertension that is associated with obesity.[21] The hypertension that is associated with pheochromocytomas may be most similar to the hypertension that is associated with preeclampsia in that in both cases there is relatively decreased plasma volume and peripheral vasoconstriction.[22] The hypertension of preeclampsia is associated with increased circulating concentrations of the N-terminus and the C-terminus of the ANF prohormone, both of which decrease into the normal range when the pheochromocytomas are removed.[23] Thus at present it would appear that in both the subjects with preeclampsia and in women with pheochromocytomas, the major contributing factor to the increased release of the N-terminus and C-terminus of the ANF prohormone is the generalized vasoconstriction, which causes a local increased volume load to the cardiac atria, which, in turn, releases both the N-terminus and C-terminus of the prohormone. In a recent essay Easterling and Benedetti[22] have advanced the hypothesis that preeclampsia is a hyperdynamic disease process that is accompanied by increased cardiac output and vasodilation early in the disease with contracted intravascular volume and elevated systemic vascular resistance as it progresses. The present study, which evaluated 15 women with preeclampsia in the third trimester of their pregnancies is compatable with the latter part of this hypothesis in that the vasoconstriction is probably the main cause of the release of these peptides. The increased circulating concentrations of the N-terminus and the C-terminus of the ANF prohormone in the third trimester of pregnancy help to explain the relative hypovolemia,[11, 17] as well as the relative hyporeninemia and hypoaldosteronism,[22] since active atrial natriuretic peptides would decrease plasma volume by the induction of natriuresis and by interstitial shift of plasma.[1, 3] The C-terminus of the prohormone has also been shown to decrease plasma renin activity and aldosterone.[13]

Although the present study did not evaluate women with preeclampsia early in their pregnancies, if the N-terminus and the C-terminus of the ANF prohormone were increasing early as a result of increased cardiac output, they would cause vasodilation as hypothesized by Easterling and Benedetti[22] since both the N-terminus[3] and the C-terminus[1, 3] contain vasodilatory peptides. A future longitudinal study of the N-terminus and the C-terminus of the ANF prohormone early and throughout preeclamptic pregnancies will document whether an early increase in these peptides may mediate the early vasodilation that is suggested in the Easterling and Benedetti hypothesis of preeclampsia.[22]

The N-terminus, the midportion of the N-terminus (prohormone ANF 31 to 67), and the C-terminus all decreased within 24 hours of delivery in the 15 subjects with preeclampsia. This is in marked contrast to a normal pregnancy in which the C-terminus[24, 25] and the N-terminus[16] increase post partum. Although the reason for this difference between patients with preeclampsia and women who have normal pregnancies is unknown, it does point to possible altered metabolism of these atrial peptides in women with preeclampsia after delivery of the infant and the placenta, which suggests that the fetus and the placenta are not involved in this altered metabolism. Whether the postpartum differences between women with normal pregnancies and women with preeclampsia in the circulating concentrations of the N-terminus and the C-terminus of the ANF prohormone relate only to marked differences in predelivery plasma volume with increased plasma volume in a normal pregnancy and decreased plasma volume in preeclampsia could not be determined from the present investigation. It is probable, however, that part of the mechanism involves a decrease in generalized vasoconstriction at delivery in women with preeclampsia with a resulting decrease in central venous pressure that would result in a decreased release of the N-terminus and the C-terminus of the ANF prohormone. In women with normal pregnancies who do not have vasoconstriction to be released at delivery, there would not be the same stimulus to decrease central venous pressure, and their peptide levels, as mentioned previously, would increase rather than decrease.

In summary, the present study demonstrates that both the circulating concentrations of the N-terminus and the C-terminus of the prohormone of ANF increase in preeclampsia. This increase is greater than that seen in the third trimester of normal pregnancies. The present study also demonstrates that both the N-terminus and the C-terminus of the ANF prohormone

decrease after delivery. This is in marked contrast to normal pregnancies in which the C-terminus and N-terminus of the prohormone increase post partum.

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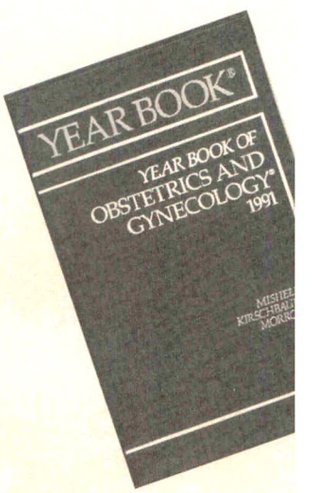


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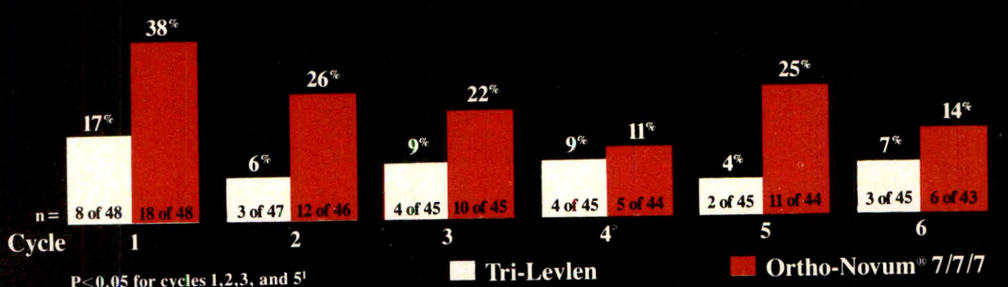
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Indications and Usage

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

Contraindications

Oral contraceptives should not be used in women with any of the following conditions: thrombophlebitis or thromboembolic disorders, a past history of deep-vein thrombophlebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estrogen-dependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pill use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long-term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of disease, namely, a ratio of the incidence of a disease among oral-contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral-contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods.

1. *Thromboembolic Disorders and Other Vascular Problems*

a. *Myocardial Infarction.* An increased risk of myocardial infarction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral-contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. *Thromboembolism.* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to four-fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

c. *Cerebrovascular diseases.* Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and nonusers, for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

d. *Dose-related risk of vascular disease from oral contraceptives.* A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogen used in the contraceptive. The amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral-contraceptive agents should be started on preparations containing less than 50 mcg of estrogen.

e. *Persistence of risk of vascular disease.* There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estrogens.

2. *Estimates of Mortality from Contraceptive Use.* One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral-contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral-contraceptive users is based on data gathered in the 1970's–but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with oral-contraceptive use after age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. Therefore, the Committee recommended that the benefits of oral-contraceptive use by healthy nonsmoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. *Carcinoma of the Reproductive Organs.* Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonusers and differences in age at start of use, has been questioned. Some studies suggest that oral-contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. *Hepatic Neoplasia.* Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users.

5. *Ocular Lesions.* There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. *Oral-Contraceptive Use Before or During Early Pregnancy.* Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing oral-contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is confirmed.

7. *Gallbladder Disease.* Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. *Carbohydrate and Lipid Metabolic Effects.* Oral contraceptives have been shown to cause glucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hyperinsulinism, while lower doses of estrogen cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see "Warnings" 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral-contraceptive users.

9. *Elevated Blood Pressure.* An increase in blood pressure has been reported in women taking oral contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestogens. Women with a history of hypertension or hypertension-related diseases, or renal disease should be encouraged to use another method of contraception. If women with hypertension elect to use oral contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension among ever-and never-users.

10. *Headache.* The onset or exacerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. *Bleeding Irregularities.* Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestogen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

Precautions

1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. (See "Warnings" 1d.) 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine-and liver-function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral-contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See "Warnings" section. 10. PREGNANCY. Pregnancy Category X. See "Contraindications" and "Warnings" sections. 11. NURSING MOTHERS. Small amounts of oral-contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

Information for the Patient

See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see "Warnings" section): thrombophlebitis, arterial thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, gallbladder disease, hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; temporary infertility after discontinuation of treatment; edema; melasma which may persist; breast changes: tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, hemolytic uremic syndrome, Budd-Chiari syndrome, acne, changes in libido, colitis, sickle-cell disease.

Overdosage

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits

The following noncontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenorrhea.

Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer.

Dosage and Administration

To achieve maximum contraceptive effectiveness, TRI-LEVLEN® tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours.

For full details on dosage and administration see prescribing information in package insert. 60627-C 5/90

Reference: 1. Data on file, Berlex Laboratories.



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Gestational age in relation to marine *n*-3 fatty acids in maternal erythrocytes: A study of women in the Faroe Islands and Denmark

Sjúrður F. Olsen, MD, MSc,[a] Harald S. Hansen, Dr.Scient.,[b] Steffen Sommer, MD,[c] Benny Jensen, Lic.Tecn.,[b] Thorkild I.A. Sørensen, Dr.Med.Sci.,[d] Niels J. Secher, MD,[c] and Petur Zachariassen, Cand.Scient.[a]

Tórshavn, Faroe Islands, and Aarhus and Copenhagen, Denmark

Gestation is longer in Faroese than Danish women, possibly because of the high intake of marine long-chain *n*-3 fatty acids that down regulates formation of prostaglandins from arachidonic acid. Polyunsaturated fatty acids were quantified in erythrocytes obtained within 2 days of delivery from randomly selected groups of 62 Faroese and 37 Danish women with an assessable gestational age. Average ratio of long-chain *n*-3 fatty acids to arachidonic acid [(3/6) ratio] was 0.73 (SD = 0.11) in Faroese women and 0.61 (SD = 0.12) in Danish women ($p < 0.0001$), corresponding to the higher intake of marine *n*-3 fatty acids in the Faroes. A 20% increase in the (3/6) ratio was associated with an increase in pregnancy duration of 5.7 days in Danish women (95% confidence interval, 1.4 to 10.1 days; $p = 0.02$) and 0.7 days in Faroese women (95% confidence interval, −2.0 to 3.3; $p = 0.6$). The hypothesized exposure-effect relationship may be weaker or absent at the higher level of exposure seen in the Faroese group. (AM J OBSTET GYNECOL 1991;164:1203-9.)

Key words: *n*-3 fatty acids, erythrocytes, gestational age, birth weight, diet

Preterm delivery is one of the most important single risk indicators of perinatal death and neonatal morbidity.[1, 2] The majority of preterm deliveries in industrialized populations remains unexplained.[3]

The Faroe Islands are located in the North Atlantic Ocean between Norway and Iceland, and the population of 47,000 has, accordingly, a substantially higher consumption of marine foods than has the population of Denmark.[4] This, combined with the findings in the Faroe Islands of increased birth weights,[5] pregnancy durations, and rate of induced deliveries,[6] together with a suggestive case history,[7] made us hypothesize that a high maternal intake of marine fat, rich in long-chain *n*-3 fatty acids, increases birth weight by prolonging duration of pregnancy.[6] The hypothesis is biologically plausible, because the dienoic prostaglandins $F_{2\alpha}$ and E_2 are promoters of parturition[8] and their formation from arachidonic acid may be down regulated by a high intake of long-chain *n*-3 fatty acids.[6, 9]

Fatty acids of the *n*-3 series cannot be synthesized de novo in the mammalian organism.[10] The amount of long-chain *n*-3 fatty acids consumed is a determinant of their level in erythrocyte phospholipids,[11-13] making it possible to rank individuals according to their intake of long-chain *n*-3 fatty acids on the basis of blood samples. Compared with questionnaire-based dietary methods, this biochemical proxy for the intake has the great advantage of being independent of the individual's memory. However, its use in epidemiologic research is hampered by the laboriousness related to the biochemical analysis and the preparation of samples before storage.

This study was undertaken to explore whether there is indeed a difference in the long-chain *n*-3 fatty acids in maternal erythrocytes in Faroese and Danish women and whether a correlation exists between long-chain *n*-3 fatty acids and pregnancy duration in one or both of these populations.

Populations and methods

Populations. The study took place at Landssjúkrahúsid, the main hospital in the Faroe Islands, and at Aarhus University Hospital in Denmark (approximately 550 and 3600 deliveries per year, respectively). Both hospitals have well-defined catchment areas, and neither of them refers women to other hospitals for delivery (except in certain cases in the Faroes, e.g., rhesus immunization, when the woman may be referred to a specialized ward in Denmark). In both areas >99% of deliveries take place in hospitals or maternity units.

From Academia Faeroensis, Faroe Islands,[a] Royal Danish School of Pharmacy, Copenhagen,[b] Aarhus University Hospital,[c] and Hvidovre University Hospital, Copenhagen.[d]
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Reprint requests: Dr. S.F. Olsen, Institute of Social Medicine, University of Aarhus, Hoegh-Guldbergsgade 8, Dk-8000 Århus C, Denmark.
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Data collection. Mothers of live-born singleton infants from the catchment areas were eligible for the study provided they had no gross pathologic conditions. The Faroese group included all eligible deliveries at the Landssjúkrahúsid during a 5-week period from October to November 1985. The Danish group included all eligible deliveries at Aarhus Kommunehospital during six randomly chosen days (24 hours) in a 5-week period from November to December 1985. Within 5 to 48 hours of delivery a peripheral venous sample was drawn. Information was obtained by a standardized interview of the mother and from hospital records, birth certificates, and records of antenatal visits. Gestational age was assessed according to an elaborate procedure designed by Hall et al.[14]; it is based primarily on menstrual data and also provides a decision scheme for stratifying deliveries according to certainty of the gestational age estimate. Assessment of gestational age was done independently of the results of biochemical analyses.

The study groups. Of the 52 Danish and 83 Faroese deliveries that took place during the data collection periods, two in each group were not eligible because of preeclampsia, rhesus immunization, insulin-dependent diabetes mellitus, or twin pregnancy. One Danish and two Faroese women refused to give blood samples, and samples from three Danish and seven Faroese women were lost. Nine Danish and 10 Faroese women had either uncertain estimate of gestational age or elective delivery; since the outcome of interest in the present study relates to the timing of spontaneous onset of delivery, these women were regarded as not eligible for analysis (however, elective postterm deliveries were included). A total of 37 Danish and 62 Faroese deliveries were included in the analysis (90% and 87% of the deliveries eligible for analysis, respectively).

Statistics. When groups were compared, either Student's t test or χ^2 test was applied. When associations between fatty acids and gestational age were investigated, linear regression was applied. Factors considered determinants of gestational age[8] that might also possibly be associated with intake of n-3 fatty acids in the study populations were treated as potential confounders: maternal prepregnant weight, height, age, parity, marital status, smoking, and employment during pregnancy (proxy for work load) (the last four factors were dichotomous). The potential joint confounding effect of these variables on the association between gestational age and the (3/6) ratio was controlled for by adding them as a group into a multiple regression model. When the term *significant* is being used, it refers to a p value of <0.05.

Biochemical material and methods

Biochemical analyses. The blood sample was immediately put on ice, and within an hour erythrocytes were isolated as described by Dodge and Phillips,[15] rinsed three times, and finally stored in 1.5 ml saline solution with 0.005% (wt/vol) butylated hydroxytoluene at −20° C. The thawed erythrocytes were resuspended and a 1 ml aliquot was taken for analysis. Then 2 ml of ice-cold methanol with 0.01% butylated hydroxytoluene was added, and the lipid extraction was carried out as described by Dodge and Phillips.[15] Methyl ester preparation and gas chromatography were performed essentially as described by Hansen and Jensen.[16] The fatty acid methyl esters in total lipids were tentatively identified by comparison of retention times with those of authentic standards (Nu-Chek Prep, Inc., Elysian, Minn.) or by calculating equivalent chain lengths of homologous series of fatty acid methyl esters.[17] For retention times where critical pairs of methyl esters were suspected, a supplementary gas chromatographic analysis was carried out on a limited number of samples. For this purpose a nonpolar column was used (30% SE-30 on 80/100 mesh Gas Chrom P, Applied Science, State College, Penn.). On the 10% SP-2330 column used for routine gas chromatography, the fatty acids 22:0 and 20:4 did not always separate, i.e., 22:0 was sometimes seen as a shoulder on the 20:4 peak. Thus these two fatty acids were calculated as one peak, referred to hereafter as 20:4 (n-6). In extracts of total lipids, 22:0 amounted to approximately 14% of the 20:4 (n-6) peak. Peak areas corresponding to butylated hydroxytoluene, to methyl heptadecanoate, and to dimethylacetals of the aldehydes 16:0, 18:0, and 18:1, or with retention times beyond 22:6, were excluded from the calculation of the peak area percentages.

Biochemical variables used in the statistical analyses. From the peak area percentages of three long-chain n-3 fatty acids, eicosapentaenoic acid, 20:5 (n-3); docosapentaenoic acid, 22:5 (n-3); and docosahexaenoic acid, 22:6 (n-3); and of arachidonic acid, 20:4 (n-6), the ratio of the sum of the three long-chain n-3 fatty acids to arachidonic acid was calculated [the (3/6) ratio]. Logarithmic transformation of this ratio, which was skewed to the right, tended to fit the linear model better.

Stability and reproducibility. No significant changes in polyunsaturated fatty acids were seen during storage for 12 months at −20° C; five assessments ($n = 4$) were made during the period, and all samples were taken at the same time from one person. The blood samples included in the study were stored for a maximum period of five months. Coefficients of variation for the long-chain fatty acids ranged from 5% to 7%.

Prepartum versus postpartum sampling. Blood samples taken from 16 pregnant Danish women at the thirty-seventh week of gestation and again within 1 day of delivery showed no significant differences in any of the four fatty acids (paired t tests), and the differences

Table I. Percentages of long-chain (*n*-3) fatty acids and arachidonic acid in erythrocytes obtained from Faroese and Danish women within 2 days of delivery*

	Faroes (mean ± SEM) (n = 62)	*Denmark (mean ± SEM) (n = 37)*	*Difference (Faroes-Denmark)*		
			Mean	*95% Confidence interval*	*p Value*
Eicosapentaenoic acid (%)	0.83 ± 0.039	0.61 ± 0.051	0.22	0.09-0.35	0.0009
Docosapentaenoic acid (%)	2.08 ± 0.059	2.08 ± 0.076	0.003	−0.19-0.19	0.97
Docosahexaenoic acid (%)	5.87 ± 0.12	4.65 ± 0.159	1.21	0.81-1.61	0.0000
Arachidonic acid (%)	12.07 ± 0.15	12.07 ± 0.19	−0.003	−0.49-0.48	0.99
Sum of long-chain *n*-3 fatty acids (%)	8.79 ± 0.17	7.35 ± 0.21	1.43	0.90-1.97	0.0000
Ratio of long-chain *n*-3 fatty acids to arachidonic acid	0.73 ± 0.014	0.61 ± 0.019	0.12	0.073-0.17	0.0000

*These data are not log transformed.

Table II. Gestational age, birth weight, and maternal characteristics in the two groups

	Faroes (mean ± SEM) (n = 62)	*Denmark (mean ± SEM) (n = 37)*	*Difference (Faroes-Denmark)*			
			Mean	*95% Confidence interval*	χ^2	*p Value*
Gestational age (days)	285.3 ± 1.3	283.4 ± 1.7	2.0	−2.2-6.2		0.3
Birth weight (gm)	3600 ± 56	3460 ± 73	140	−44-324		0.1
Age (yr)	26.8 ± 0.68	27.6 ± 0.88	−0.9	−3.1-1.3		0.4
Height (cm)	164.4 ± 0.7	165.6 ± 0.9	−1.3	−3.5-0.9		0.3
Weight (kg)	58.7 ± 1.2	59.4 ± 1.6	−0.7	−4.7-3.3		0.7
Employment (total hr)	861 ± 76	732 ± 99	128	−122-378		0.3
Primiparous (%)	38	60	—	—	4.01	0.04
Single (%)	14	15	—	—	0.02	0.9
Employed during pregnancy (%)	79	68	—	—	1.61	0.2
Cigarettes/day during pregnancy (%)						
0	66	68				
1-5	8	8				
6-15	23	16				
≥16	3	8	—	—	1.57	0.7

were not correlated with the duration from first to second sampling, which ranged from 3 to 34 days, with an average of 17 days. The difference between prepartum and postpartum levels of the (3/6) ratio was 0.0022 (95% confidence interval, −0.032 to 0.036; $p = 0.9$).

Results

Eicosapentaenoic acid and docosahexaenoic acid occurred in significantly higher percentages in the Faroese group, whereas docosapentaenoic acid and arachidonic acid showed similar values in the two groups (Table I). The sum of long-chain *n*-3 fatty acids and the ratio of this sum to arachidonic acid [the (3/6) ratio] were significantly greater in the Faroese than in the Danish group ($p < 0.0001$). Average gestational age was 2.0 days longer and birth weight was 140 gm higher in the Faroese group than in the Danish group, but the differences were not significant (Table II). There were significantly more primiparous women in the Danish group, but maternal age, prepregnant weight, and height and the proportions of women who were single, who smoked, or who were employed during pregnancy were similar for both groups (Table II). Including women with uncertain lengths of gestation and with elective deliveries did not alter these conclusions.

The relation between gestational age and the (3/6) ratio is shown in Fig. 1. In the simple regression of gestational age on the log-transformed (3/6) ratio, a positive and borderline significant association was seen in the separate analysis of Danish women but not in that of Faroese women (Table III, models 1 and 3).

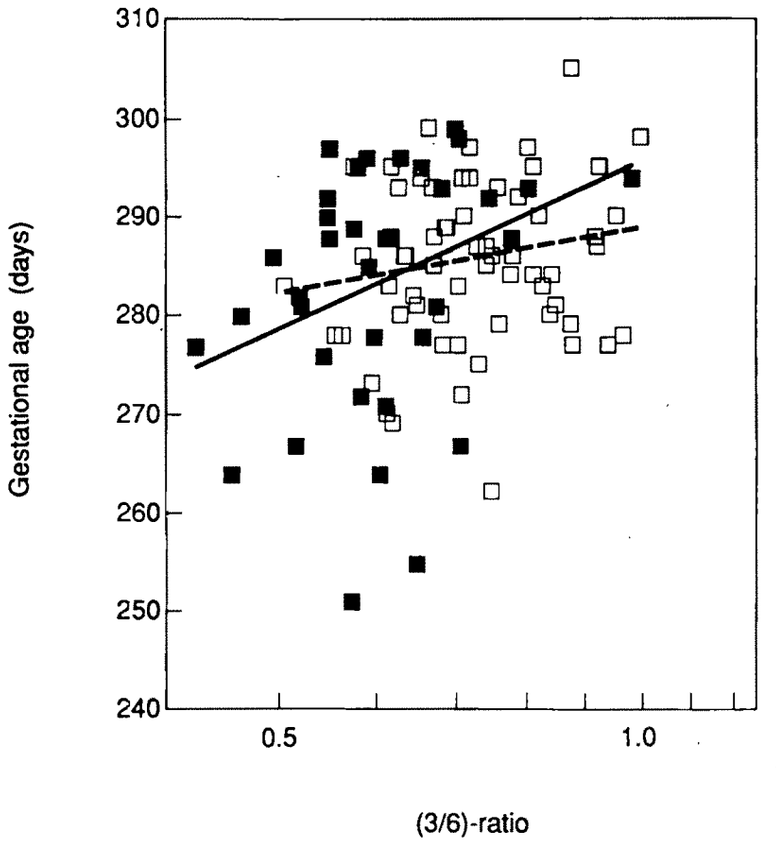


Fig. 1. Gestational age and (3/6) ratio. Danish and Faroese deliveries are indicated by *black* and *white squares,* respectively. Fitted lines from regression analyses of Danish and Faroese groups are given as *solid line* and *dashed line,* respectively. The x axis is logarithmically transformed. The equation of the Danish fitted line is: Gestational age = 55.1 × $\log_{10}$(3/6 ratio) + 295. The equation of the Faroese fitted line is: Gestational age = 18.8 × $\log_{10}$(3/6 ratio) + 288.

Controlling for potential confounders strengthened the association seen in Danish women (model 2 vs 1) and weakened that seen in Faroese women (model 4 vs 3).

To control for site of origin in the analysis of the combined groups, the variable *Residence* was used. Neither in the model with potential confounders nor in that without was there significant interaction (p = 0.07 and 0.2, respectively) between the (3/6) ratio and *Residence,* as expressed by the product term: (3/6) Ratio × Residence (Table III, models 5 and 6). In a model with no interaction assumed, only minor changes were seen in the regression coefficient of the (3/6) ratio when potential confounders, including the *Residence* variable, were eliminated from the model (Table III, models 7 to 9).

The associations between gestational age and the sum of the long-chain *n*-3 fatty acids were generally weaker than the associations between gestational age and the (3/6) ratio, but similar trends were seen. Thus the correlation coefficient between gestational age and the log-transformed sum was 0.22 (p = 0.2) in the Danish group, 0.07 (p = 0.6) in the Faroese group, and 0.17 (p = 0.1) in the combined groups, whereas the corresponding correlation coefficients between gestational age and the log-transformed (3/6) ratio were 0.33 (p = 0.05), 0.15 (p = 0.2), and 0.25 (p = 0.01), respectively.

Comment

The hypothesis of this study, i.e., that dietary long-chain *n*-3 fatty acids prolong gestation, is based on the contention that long-chain *n*-3 fatty acids inhibit the formation of the dienoic prostaglandins from their obligate precursor, arachidonic acid.[6, 9, 10] Thus the ratio of long-chain *n*-3 fatty acids to arachidonic acid [the (3/6) ratio] seems to be the most reasonable single biochemical measure of exposure. The average (3/6) ratio of Faroese women was significantly greater than that of Danish women; this was to be expected[11-13] because the intake of marine fat is substantially higher in the Faroes.[4]

A positive association was seen between pregnancy

Table III. Multiple regression analyses of gestational age on the $\log_{10}$-transformed (3/6) ratio alone and together with other explanatory variables*

Variables included in the model	*Regression coefficient (days/variable unit)*		*1 df*	*2 df*	*Correlation coefficient (multiple R)*
	Mean ± SEM	*p Value*			
Denmark (n = 37)					
Model 1					
(3/6) Ratio alone	55.1 ± 27.1	0.05	1	35	0.325
Model 2					
(3/6) Ratio	72.5 ± 28.1	0.02	8	28	0.635
7 Potential confounders	—	—			
Faroes (n = 62)					
Model 3					
(3/6) Ratio alone	18.8 ± 15.5	0.2	1	60	0.155
Model 4					
(3/6) Ratio	8.16 ± 16.9	0.6	8	53	0.365
7 Potential confounders	—	—			
Faroes and Denmark (n = 99)					
Model 5					
(3/6) Ratio	12.8 ± 19.3	0.5	10	88	0.414
Residence (Faroes = 0; Denmark = 1)	10.8 ± 6.07	0.08			
(3/6) Ratio × Residence	54.9 ± 30.0	0.07			
7 (Other) potential confounders	—	—			
Model 6					
(3/6) Ratio	18.8 ± 18.6	0.3	3	95	0.283
Residence (Faroes = 0; Denmark = 1)	7.45 ± 5.85	0.2			
(3/6) Ratio × Residence	36.3 ± 28.8	0.2			
Model 7					
(3/6) Ratio	35.6 ± 14.9	0.02	9	89	0.373
Residence (Faroes = 0; Denmark = 1)	0.594 ± 2.44	0.8			
7 (Other) potential confounders	—	—			
Model 8					
(3/6) Ratio	33.9 ± 14.2	0.02	2	96	0.254
Residence (Faroes = 0; Denmark = 1)	0.685 ± 2.32	0.8			
Model 9					
(3/6) Ratio alone	31.9 ± 12.4	0.01	1	97	0.252

*The seven potential confounders are listed in the Statistics section. The regression coefficient of the (3/6) ratio is given in days per unit change of the $\log_{10}$-transformed ratio, with one unit equaling a 10 times increase in the ratio; multiplying the regression coefficient by $\log_{10}(1.5)$ or by $\log_{10}(1.2)$ changes the unit of the regression coefficient to days per 50% increase or per 20% increase, respectively, in the ratio. *df*, Degrees of freedom. Further details about the analyses are available from the authors.

duration and the (3/6) ratio in the Danish group, whereas no association could be detected in the Faroese group. Thus, after allowances were made for seven potential confounders, a 20% increase in the (3/6) ratio was estimated to be associated with an increase in pregnancy duration of 5.7 days in Danish women (95% confidence interval, 1.4 to 10.1 days; $p = 0.02$) and 0.7 days in Faroese women (95% confidence interval, −2.0 to 3.3; $p = 0.6$) (Table III, models 2 and 4). In theory, three possible explanations may be considered. First, the true relationship is being reflected in the association seen in the Danish group, and the absence of association in the Faroese group is due to statistical error (type II) or to some source of bias not controlled for, such as saturation of phospholipid fatty acid pools in the Faroese women.[18] Second, there is no causal relationship between maternal *n*-3 fatty acid intake and pregnancy duration, and the association seen in the Danish group is due to statistical error (type I) or bias. Third, the results from both groups represent the truth, i.e., the associations differ in the two populations; indeed this possibility was foreseen as a subordinate, a priori hypothesis because, even if the main hypothesis of an effect of *n*-3 fatty acids on gestational age were true, the exposure distribution of one of the two populations could be below or above the working range of the exposure-effect relationship. However, the difference between the estimates of association (measured by the term of interaction in the combined analysis) did not reach the conventional level of statistical significance. Exploring whether the associations truly do differ in the two populations and refining the model by, for

instance, searching for threshold effects would require a new study with a larger sample size.

According to our previous studies that were based on birth certificates from much larger numbers of women than in the present study, average birth weight of live-born babies in the Faroes was highest among 33 geographically defined populations.[5] Average birth weight and gestational age of live-born singleton infants of primiparous women were 194 gm higher and 3.6 days longer, respectively, in the Faroes than in Denmark.[6] Women delivered after 41 completed weeks of gestation were 1.9 times more frequent in the Faroes and those delivered before 37 completed weeks were 1.8 times more frequent in Denmark; these differences could not be attributed to a difference in induction practice because the proportion of induced deliveries was highest in the Faroes.[6] Information from birth certificates from the two populations is comparable, as the forms in use are identical and computerized at the same office. Practically all doctors and midwives in the Faroes have been trained in Denmark, ensuring similar obstetric procedures and terminology.

The average (3/6) ratio was about 20% higher in the Faroese than in the Danish group. As interaction between the *Residence* variable and the (3/6) ratio was nonsignificant, the model assuming no interaction may be used to estimate the "average effect"[19] in the two populations. In this model (Table III, model 7) a 20% increase in the (3/6) ratio was accompanied by a 2.8-day increase in pregnancy duration (95% confidence interval, 0.5 to 5.2 days; $p = 0.02$). Although the wide confidence interval around the estimate should be kept in mind, it is compatible with the difference of 3.6 days observed between the two populations in our previous study[6] referred to above.

To our knowledge, only one controlled trial has been published where *n*-3 fatty acids were given to pregnant women. During 1938 to 1939 in London, >5000 women were alternately allocated to receive either no supplement or a supplement with vitamins, minerals, and halibut liver oil.[20] The trial, which has recently been reviewed,[21] was of comparatively high standard.[21] A significantly smaller proportion of pregnancies with short durations was seen in the supplemented group. It is not possible to tell which of the components of the treatment caused the effect, but it is striking that this effect has not been demonstrated convincingly in later controlled trials undertaken with vitamins and minerals given in the same amounts as in the London trial.[21]

Grundy et al.[22] found that women of Asian origin had shorter average pregnancy duration and fewer postterm and induced deliveries than Caucasian women, although they were living in the same area of London, were delivered at the same hospital, had similar distributions as to parity and social class, and had a lower proportion of smokers. No information was provided on the nutritional habits of this Asian group, but McKeigue et al.[23] found a high proportion of vegetarians and a low fish intake in an Asian group that was defined similarly as to country of origin and where they were living in London (mainly Hindus); this was supported by the finding of lower levels of *n*-3 fatty acids and higher levels of *n*-6 fatty acids in plasma cholesterol esters of men from this Asian group than in those of Caucasian men in the United Kingdom. Although alternative explanations cannot be excluded, the finding of shorter gestations in Asian women is apparently in agreement with our hypothesis.

A greater proportion of early deliveries was found in rats supplemented with arachis oil, which is high in *n*-6 fatty acids, than in rats supplemented with fish oil, which is high in *n*-3 fatty acids; this finding is also compatible with the basic idea that a high ratio of *n*-3 to *n*-6 fatty acids in the diet postpones delivery.[24]

In a review on nutrition and pregnancy duration, Kristal and Rush[25] stated that there is little to suggest much of an association between diet and duration of gestation among adequately nourished women in developed countries. The results from our study indicate that some previously unrecognized aspects of the diet may be of significance. However, further studies are needed to explore whether the statistical association seen in the Danish group truly does reflect a causal relationship between intake of marine *n*-3 fatty acids and gestational age. A trial assessing the effect of marine *n*-3 fatty acids on pregnancy duration seems justified. It should preferably be done on a multicenter basis, permitting assessment of the potential preventive effects on premature delivery and on other adverse pregnancy outcomes.[26]

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Recurrent failure of in vitro fertilization: Role of the hemizona assay in the sequential diagnosis of specific sperm-oocyte defects

Sergio Oehninger, MD,[a] Anibal A. Acosta, MD,[a] Lucinda L. Veeck, MLT,[a] Robert Brzyski, MD, PhD,[a] Thinus F. Kruger, MD,[b] Suheil J. Muasher, MD,[a] and Gary D. Hodgen, PhD[a]

Norfolk, Virginia, and Tygerberg, Republic of South Africa

The results of predictive fertilization bioassays (hemizona assay, hamster ova–human sperm penetration assay), in vitro fertilization treatment, fertile donor cross-match tests with either sperm or oocytes, and oocyte micromanipulation for assisted fertilization were used to establish a pathophysiologic diagnosis in cases of recurrent failed fertilization in vitro. Disorders of sperm function manifested at the level of zona binding, zona penetration, oolemma fusion, and pronuclear decondensation as well as oocyte anomalies were considered to represent the specific gamete defects that led to abnormal sperm-oocyte interactions (i.e., failed fertilization). Our findings show that the sequential application of bioassays can elucidate specific sperm or oocyte defects that characterize functional abnormalities among sperm populations. (AM J OBSTET GYNECOL 1991;164:1210-5.)

Key words: Failed fertilization, sperm function disorders

In the Norfolk in vitro fertilization (IVF) and embryo transfer program, failure of fertilization of mature, preovulatory oocytes has been observed in 8.9% of patients and 5.7% of cycles.[1] Certain aberrations of the fertilization process have been recognized including polyspermia in 9.0% of preovulatory oocytes and delayed fertilization in 1.8% of cycles.[1,2] Whereas these abnormalities may point to inadequately timed oocyte insemination or inappropriate in vitro culture conditions, it is more likely that gamete defects are contributory. Whatever the underlying cause(s), such factors combine to have a significant negative impact on the overall success of IVF treatment.

An elusive goal remains in the search for a test that will accurately predict IVF outcome. In a previous article,[3] we reported that sperm morphology evaluated by strict criteria is a useful predictor of IVF results. Motility indexes are not indicators unless progressive motility is <15% or when <1.5 million motile sperm are obtained after swim-up separation.[4] Thus, tests designed to evaluate functional sperm (and oocyte) normalcy relative to IVF outcome become relevant so that patients can be counseled accordingly. In this regard, the homologous hemizona assay,[5] a zona penetration assay,[6] and the heterologous zona-free hamster ova–human sperm penetration assay[7] constitute differential bioassays that evaluate tight sperm-oocyte binding, zona penetration, oocyte fusion, and decondensation in sequence.

Here, we have studied IVF treatment failure in four couples to establish a pathophysiologic diagnosis for specific functional defects of sperm-oocyte interactions during the fertilization process and to develop a serial diagnostic scheme for cases of recurrent fertilization failure in vitro. It is hoped that the information gained can be useful in the selection of optimal therapy, such as modification of standard IVF methods or micromanipulation of oocytes for assisted fertilization.[8-12]

Material and methods

Patients, semen analysis, and endocrine profile. Four couples participating in the Norfolk IVF program during seven treatment cycles constituted the study group. Semen samples (at least two collections) were evaluated for sperm concentration, percentage of motility, velocity, and linearity (Cellsoft Semen Analyzer, Cryo Resources, Ltd., New York), percentage of normal forms (strict criteria), bacteriology, and immunology (direct immunobead test), according to published methods.[1,3,4,9] Serum endocrine evaluations were performed as described.[4] The male partners in all couples

From The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School,[a] and Department of Obstetrics and Gynecology, Tygerberg Hospital.[b]
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Reprint requests: Sergio Oehninger, MD, The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, 855 West Brambleton Ave., Suite B, Norfolk, VA 23510.
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had normal physical examination results, negative results of bacteriologic and immunologic evaluations, and normal serum endocrine profile results.

In vitro fertilization and embryo transfer procedures. Ovarian stimulation protocols, semen processing for IVF, oocyte insemination, and embryo culture and transfer were performed according to established guidelines.[1, 2] Because of the abnormal sperm parameters observed in their original semen samples and a history of failed fertilization during one or more IVF treatment cycles, each oocyte was inseminated with a 10-fold higher sperm per milliliter concentration (0.5 to 2.0 $\times$ 10^6 motile sperm after swim-up separation).[9] In all cases, the total number of motile spermatozoa recovered after swim-up was $>1.5 \times 10^6$. No attempts were made to remove the cumulus oophorus either mechanically or enzymatically in any of the cases.

Cross-match test in IVF. When patients agreed and after their written consent was obtained, cross-match tests were performed as follows: (1) the wife's oocytes (metaphase II or immature, in vitro–matured metaphase II) were inseminated with frozen-thawed donor semen (fertile controls) at 500,000 motile sperm per milliliter per oocyte, and (2) donor oocytes (prophase I matured to metaphase II in vitro) were inseminated with the husband's semen at the same concentration. If fertilization occurred, as judged by normal pronuclear formation, further development was not allowed, or culture was continued until transfer, as previously decided by the couple.

Sequential specific sperm defect analysis

Tight binding and penetration of the zona pellucida. We have used the hemizona assay to test for a defective sperm-zona interaction.[5, 13-15] Human oocytes harvested from surgically removed ovaries and stored in salt solution were used in the study.[5, 13] In each study, one hemizona was coincubated with the patient's swim-up processed semen, whereas the matching hemizona was incubated with donor's sperm (fertile control). Tight binding was assessed after 4 hours of gametes coincubation.[5]

Acrosome reaction and oolemma fusion. By use of the hamster test or sperm penetration assay, we evaluated the ability of the human sperm to undergo the acrosome reaction and fuse with the denuded hamster oocyte membrane.[7, 8] Biggers, Whitten, and Whittingham medium was used for incubation supplemented with 3% bovine serum albumin in a 6-hour incubation protocol.[8] All control semen samples (fertile donors) penetrated >20% of eggs.

Sperm decondensation and pronuclei formation. Oocyte micromanipulative procedures for assisted fertilization were accomplished following the methods of Lanzendorf et al.[16] (microinjection of a single spermatozoon into the cytoplasm of the oocyte) or Cohen et al.[17] (partial zona dissection) after Institutional Review Board approval and written consent from the patients were obtained.

Results

Couple 1. This couple had primary infertility of 10 years' duration. The wife and husband were 33 and 34 years old, respectively. The wife's workup showed stage II endometriosis (revised American Fertility Society classification).[18] Semen analysis revealed 52 million sperm per milliliter, motility 37%, velocity 33 μm/sec, linearity 5.2, 49 million motile sperm per ejaculate, 0% normal forms, and an 11% morphology index (poor prognosis pattern, defined as <4% normal forms and morphology index <30%),[3] an hemizona assay index of 0 (number of patient's tightly bound sperm per control $\times$ 100, normal = 100),[5] and sperm penetration assay (test vs control) 0% vs 32%. In the two IVF attempts, no fertilization occurred among four mature (metaphase II) oocytes. The couple chose not to do a cross-match test and not to have oocyte microsurgical procedures performed (Tables I and II).

Couple 2. This couple had primary infertility of 5 years' duration. The wife and husband were 38 and 37 years old, respectively. The wife's workup revealed a tubal factor (failed bilateral fimbrioplasty). The husband's semen analysis showed sperm concentration 59 million per milliliter, motility 40%, velocity 26 μm/sec, linearity 7.0, 104 million motile sperm per ejaculate, 6% normal forms, 42% morphology index (good prognosis pattern),[3] hemizona assay index of 75, and sperm penetration assay 27% vs 28% (Tables I and II).

Four IVF attempts at different institutions resulted in no fertilization of multiple eggs. Two IVF attempts were made in Norfolk. In the first attempt, 11 preovulatory (metaphase II) oocytes were recovered. Nine metaphase II and three immature (prophase I) oocytes were obtained in the second IVF attempt.

In the first IVF attempt, no fertilization occurred in seven metaphase II oocytes; one of four microinjected metaphase II eggs fertilized and was transferred at the two-cell stage after incubation for 40 hours. In the second IVF cycle, no fertilization was observed among four metaphase II oocytes. One of three microinjected oocytes fertilized and was transferred at the pronuclear stage. Neither of the two preovulatory, metaphase II eggs subjected to partial zona dissection fertilized. Two immature (prophase I) eggs matured in vitro were inseminated with donor sperm; both fertilized but were not transferred. In addition, one donor egg failed to fertilize after insemination with husband's sperm (Table II).

Couple 3. This couple had infertility of 3 years' du-

Table I. Sperm analysis in couples with recurrent failed fertilization

Patient	Sperm parameters				
	Concentration ($\times 10^6$ *ml*)	*Motility* (%)	*Velocity* (μm/sec)	*Linearity*	*Normal morphology* (%)*
1	52	37	33	5	0
2	59	40	24	7	6
3	143	71	36	6	4
4	31	48	40	6	6

*Strict criteria.

Table II. Results of hemizona assay, sperm penetration assay, IVF, oocyte micromanipulation, cross-match tests, and embryo transfer in cases of failed fertilization

Patient	IVF cycle	Hemizona index	SPA (%)*	Fertilization in IVF	Fertilization after microinjection	Fertilization after partial zona dissection	IVF cross-match		No. of embryos transferred
							Donor sperm	Donor eggs	
1	a	0	32 vs 0	0/3	—	—	—	—	—
	b	—	—	0/1	—	—	—	—	—
2	a	75	28 vs 27	0/7	1/4	—	—	—	1 (2 cells)
	b	—	—	0/4	1/3	0/2	2/2	0/1	1 (pronuclear stage)
3	a	65	100 vs 0	0/11	4/4	—	—	—	4 (2 cells)
	b	—	—	0/7	2/6	0/2	3/3	0/2	3 (4 cells)
4	a	108	25 vs 20	1/7	—	—	—	1/1	2 (4 cells)

SPA, Sperm penetration assay.
*Control vs test.

ration. The wife and husband were 29 and 32 years old, respectively. The wife's workup was normal. Semen analysis results were sperm concentration 143 million per milliliter, motility 71%, velocity 36.4 μm/sec, linearity 6.2, 369 million motile sperm per ejaculate, 4% normal forms, 32% morphology index (good prognosis pattern),[3] hemizona assay index of 65 (Tables I and II), and sperm penetration assay 0% vs 100% (Tables I and II).

Three IVF attempts in another treatment program resulted in the collection of multiple eggs with no fertilization. Two IVF attempts were made in Norfolk. In the first IVF cycle, no fertilization was observed among 11 metaphase II oocytes. Four other metaphase II eggs were microinjected; all displayed normal pronuclear formation and were transferred at the two-cell stage at 40 hours.

In the second IVF cycle, no fertilization occurred with seven metaphase II oocytes. Three other preovulatory metaphase II eggs were inseminated with donor semen. All three were fertilized and transferred at the four-cell stage; two metaphase II oocytes were microsurgically manipulated by partial zona dissection, but none fertilized. Two of six prophase I eggs that were microinjected at the metaphase II stage fertilized and were cryopreserved at the pronuclear stage with 1,2-propanediol and remain in storage. In addition, none of two donor eggs was fertilized by the husband's sperm, although numerous sperm were observed at the zona (Table II).

Couple 4. This couple had primary infertility of 3 years' duration. The wife and husband were 31 and 30 years old, respectively. The wife's workup revealed severe endometriosis (revised American Fertility Society classification, stage IV). The semen analysis showed a sperm concentration of 31 million per milliliter, motility 48%, velocity 40 μm/sec, linearity 6.0, 83 million motile sperm per ejaculate, 6% normal forms, morphology index 30% (good prognosis pattern), hemizona assay index 108, and sperm penetration assay 20% vs 25% (Tables I and II).

Three attempts at different centers resulted in no fertilization of multiple oocytes. In one attempt, delayed fertilization of two metaphase II oocytes occurred with subsequent embryo transfer but no pregnancy. One IVF attempt was carried out in Norfolk with seven metaphase II oocytes retrieved. No fertilization occurred in six oocytes; delayed fertilization was observed in one egg.

Comment

Despite the limited data available, this study shows the feasibility of establishing a pathophysiologic diagnosis in cases of recurrent failed IVF treatment by sequential clinical and laboratory analysis. Our findings indicate that application of the hemizona assay, sperm

penetration assay, standard IVF, donor gamete crossmatching and assisted fertilization techniques, permit identification of specific sperm-oocyte dysfunctions. The order of progression may be important because those sperm defects that prevent tight binding of sperm to the zona pellucida or its penetration will negate subsequent functional tests. Accordingly, the hemizona assay should be applied first. Men whose specimens show adequate tight binding and penetration under hemizona assay conditions can be examined next for acrosome reaction, oolemma fusion, decondensation, and pronuclear formation in sequence.

Couple 1 represents a severe male factor case with a high percentage of abnormal sperm forms (mainly morphologic head defects; poor prognosis pattern).[3, 9] Morphology alone predicted a poor IVF outcome; despite increasing sperm insemination concentration by 10-fold, fertilization did not occur.[9] In addition, the hemizona assay, with an index of 0, clearly identified this patient at high risk of failed fertilization.[14] Because we have observed previously a good correlation between the incidence of abnormal sperm forms and poor binding capacities in the hemizona assay, this case represents a lack of fertilization because of a functional defect disorder in this first step.[14, 19] Because of an abnormality in sperm-zona binding, micromanipulative procedures that assist fertilization by bypassing the zona deserve consideration as a therapeutic approach.[11, 16, 17] This patient also showed negative sperm penetration assay results, signaling multiple sperm defects. If poor sperm penetration assay results are a result of abnormalities in sperm-egg fusion, then microinjection of a spermatozoon into the oocyte cytoplasm might be beneficial. However, if poor sperm penetration assay results are because of an abnormal sperm decondensation (sperm-oocyte disorder) then the remedy may be to consider donor gametes.

In couple 2, female diagnosis was mechanical (tubal) infertility; male diagnosis was asthenoteratospermia. Unlike the previous case, sperm binding to the zona was satisfactory; in addition, sperm performance in the sperm penetration assay was adequate, implying normal capacitation, acrosome reaction, fusogenic ability, and decondensation into zona-free eggs. Also pronuclei formation occurred after microinjection. However, neither the wife's nor the donor's oocytes fertilized in vitro. That donor semen fertilized some of the wife's eggs is evidence of adequate oocyte quality. These results point to a defect in the ability of the sperm to penetrate the zona pellucida as the subjacent cause of failed fertilization. A zona penetration assay[6] and electron microscopic evaluation[15] of the unfertilized oocytes may further clarify this diagnosis. The mechanisms of sperm penetration of the zona are not completely understood but probably require receptor-ligand events, adequate motility patterns, and enzymatic mechanisms. Subtle disorders in any of these mechanisms may contribute to impaired zona penetration.

Considered alone, hemizona assay results for patient 2 produced a false-positive result, as did sperm penetration assay results. We have previously reported[7] that negative sperm penetration assay results may not reliably correlate with IVF outcome (high incidence of false-negative results); however, we have observed a positive correlation between a good sperm penetration assay performance and successful IVF. Also, the fact that in the second patient's transfer the preembryo was at the pronuclear stage suggests delayed or arrested development after oocyte microinjection. We have observed a lower cleavage rate in microinjected eggs as compared with the usual cleavage rate after IVF, perhaps implying a compromised developmental potential of some of these micromanipulated oocytes either because the sperm is inferior or the method is flawed.

Couple 3 had no identifiable female abnormalities; male clinical diagnosis was teratospermia. The morphology score was on the verge of poor with modest hemizona assay index. That no penetration was observed in the sperm penetration assay and no fertilization occurred in IVF with the wife's or donor's eggs suggest a severe male factor. The fact that the wife's oocytes were successfully fertilized by donor sperm confirms the absence of a female factor and supports the probability of adequate oocyte quality. Because microinjected sperm decondensed and formed pronuclei, we suggest that those sperm functions (or oocyte and sperm functions) were satisfactory, thus pointing to an abnormality at the level of sperm-egg fusion (spermatozoon-oolemma interactions). This opinion also fits with the finding that no fertilization occurred after an opening was mechanically created in the zona. Perhaps a sperm receptor defect or an abnormal acrosome reaction is the underlying cause.

The hemizona assay is an internally controlled, homologous bioassay with good predictive value for IVF results.[14, 19] Earlier, we reported positive and negative predictive values of 95% and 83%, respectively, in anticipating IVF outcome with the hemizona assay.[14] However, the findings in couples 2 and 3 represent false-positive results. According to current standards, an hemizona assay index value of ≥ 36 correlates positively with successful fertilization in IVF, although scores nearer 100 are more desirable.[14, 20] As we indicated, it is important to realize that other steps after the binding of sperm to the zona are necessary for fertilization and viable embryo development. Accordingly, such false-positive results are expected but have been infrequent in our experience. Because hemizona assay results are overall good predictors of IVF outcome,[14, 19] it may well be that sperm binding to the zona pellucida is one of

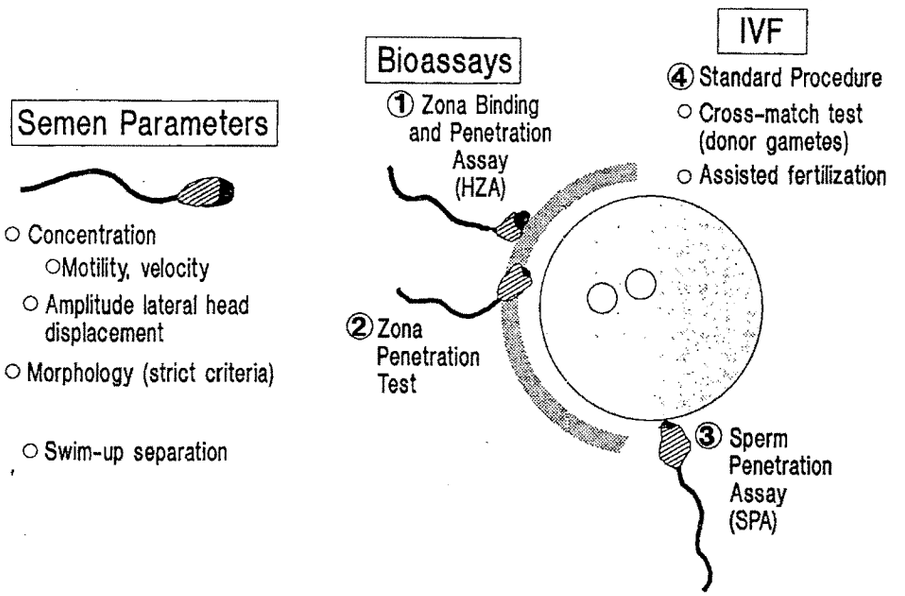


Fig. 1. Diagnostic scheme to establish a step-wise progressive diagnosis in cases of recurrent failed IVF.

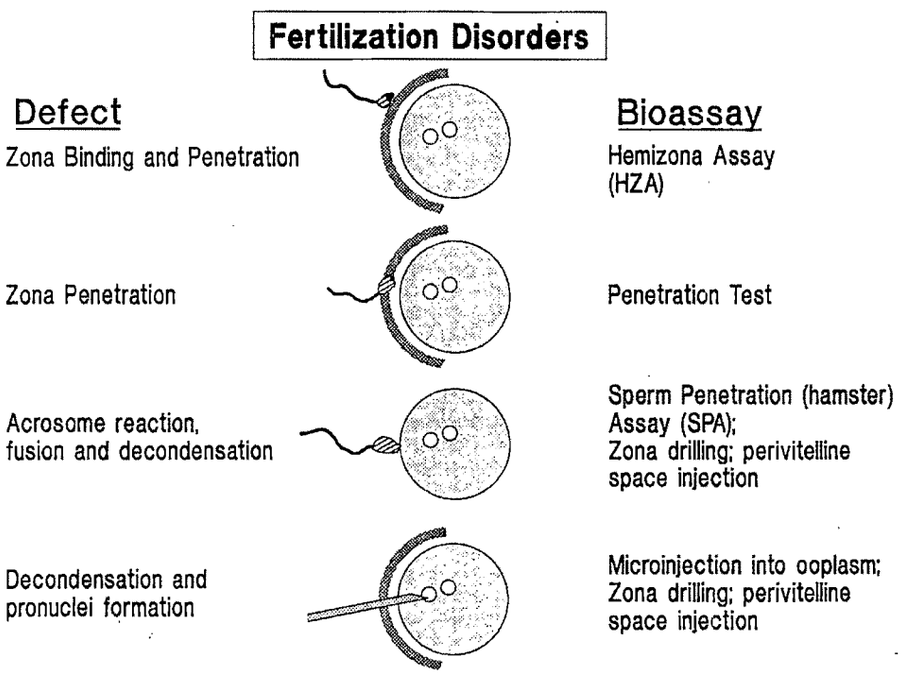


Fig. 2. Sequential analysis of specific sperm defects in cases of recurrent failed fertilization in vitro.

the more critical steps in achieving fertilization, and that fertilization failures that occur after zona-binding stages are less common or correlated to observations in zona binding.

Couple 4 represents an apparent case of poor oocyte quality. Overall, the semen sample was of acceptable quality except for teratospermia and within a good prognosis pattern. The results of the hemizona assay and sperm penetration assay were quite favorable. In addition, the husband's sperm successfully fertilized a donor egg (male cross-match test). Although the couple did not accept a female cross-match test (wife's eggs inseminated with donor sperm), we suggest that multiple failures and one delayed fertilization were a

result of oocyte dysfunction(s).[2] We have observed several oocyte defects in cases of failed and delayed fertilization.[1,2] Whether the poor oocyte quality is intrinsic or whether it is iatrogenic (stimulation-induced) remains to be investigated. Oocyte dysfunctions responsible for fertilization disorders might reside in the zona pellucida, oolemma, or ooplasm (sperm decondensation is estimated to be largely an oocyte function dependent on breakage of disulfide bonds of the condensed head by glutathione or glutathione-like substances).[21] In special circumstances, electron microscopic studies may help to determine the fate of the sperm cells whether in the zona, perivitelline space, or inside the oocyte.

The limited number of couples studied here must be acknowledged, but serves us well in showing that bioassays of fertilization potential, IVF treatment, gamete cross-match tests during IVF, and assisted fertilization techniques contribute sequentially to achieving full defects analysis on sperm-oocyte interactions. Thus, a diagnostic scheme for cases of recurrent fertilization failure can be applied as illustrated in Figs. 1 and 2. Although much more research and a larger patient series is needed to accomplish full diagnosis of gamete defects, ultimately a solid diagnosis remains the optimal course to development of effective male factor infertility treatment.

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Umbilical vein administration of oxytocin for the management of retained placenta: Is it effective?

Maarten G.P. Huber, MD,[a] Hajo I.J. Wildschut, MD, PhD,[a, d] Kees Boer, MD, PhD,[a] Gunilla Kleiverda, MD,[b] and Frans J. Hoek, PhD[c]

Amsterdam, The Netherlands, and Bristol, United Kingdom

In a multicenter randomized controlled trial involving 220 women with retained placenta no beneficial effects could be established of intraumbilical vein administration of 10 IU of oxytocin in 20 ml of saline solution. A reduction was not gained in the rate of manual removal of the placenta and there was no decrease in the amount of blood loss. Oxytocin only induced a minor shortening of the median time interval from administration to the spontaneous expulsion of the placenta as compared with a placebo injection. Maternal serum α-fetoprotein levels before and after intraumbilical vein injection did not show evidence of fetomaternal transfusion. (Am J Obstet Gynecol 1991;164:1216-9.)

Key words: Retained placenta, labor third stage, randomized controlled trial, oxytocin, umbilical vein, α-fetoprotein, fetomaternal transfusion

After vaginal delivery, a retained placenta forms a substantial risk to maternal health.[1] Manual removal of the placenta is not without danger. Any other treatment that might reduce the need to perform this procedure is of interest. The intraumbilical injection of oxytocin to induce the spontaneous expulsion of the retained placenta was first published by Senatore[2] in 1953. In 1983, Golan et al.[3] published the method in this Journal. They injected 20 ml of saline solution that contained 10 IU of oxytocin in 10 parturients with retained placenta. Within minutes this resulted in the spontaneous expulsion of the placenta in all the women. This publication prompted us to carry out a randomized, double-blind, controlled study in women with retained placenta to evaluate the efficacy of this promising treatment.

Material and methods

Between Feb. 1, 1986, and Jan. 31, 1989, parturient women from eight participating hospitals were enrolled in the study. Inclusion criteria were similar to those formulated by Golan et al.[3]: retention of the placenta for 30 minutes or more after the vaginal delivery of a singleton infant of at least 28 weeks' gestation (196 days).

After informed consent was given, for each woman a box was taken in numeric order from a stock of identical white sealed boxes that bore a serial code, by which randomization occurred in three groups: an oxytocin-treated group (group 1), a placebo-treated group (group 2), and a group of nontreated control subjects (group 3). For this a blocking procedure[4] was used whereby the number of patients in each group was balanced in small blocks of a fixed size ($n = 6$). The boxes contained a questionnaire, an unmarked ampule, and instructions for its use. The code was held by M.H. and was broken after completion of the trial.

In the questionnaire on obstetric events, maternal age refers to the age of the mother at delivery and parity as defined as the number of previous pregnancies that lasted >16 weeks.

The intervention protocol did not interfere with conventional policies to expel the placenta, but prescribed whether to inject the content of the ampule intraumbilically between 30 and 60 minutes after delivery after the addition of saline solution to a volume of 20 ml. In the last instance (not to inject, i.e., group 3) the treatment was obviously not blinded to the clinician. In the first instance the unmarked ampule either contained 10 IU oxytocin (Piton-S, Organon, Oss, The Netherlands) in 1 ml saline solution (group 1) or only 1 ml of saline solution (group 2). After mixing, the solution was injected into the umbilical vein, after which a hemostat was placed on the umbilical cord distal to the site of injection. To distribute the solution into the placenta the cord was "milked" backward after injection of the solution.

In group 3 an ampule containing 1 ml saline solution

From the Department of Obstetrics and Gynaecology, Academisch Medisch Centrum, University of Amsterdam,[a] the Department of Obstetrics and Gynaecology, Burgerziekenhuis,[b] the Department of Clinical Chemistry, Academisch Medisch Centrum, University of Amsterdam,[c] and the Institute of Child Health, Division of Epidemiology, University of Bristol.[d]

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Reprint requests: K. Boer, MD, PhD, Department of Obstetrics and Gynaecology, Academisch Medisch Centrum H4N-205, Meibergdreef 9, Amsterdam, The Netherlands 1105 AZ.

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Table I. Characteristics of women with retained placenta

	*Oxytocin (n = 72)**		*Placebo (n = 69)**		*Control (n = 59)**	
	Mean, proportion	*95% CI*	*Mean, proportion*	*95% CI*	*Mean, proportion*	*95% CI*
Maternal age (yr)	28.3	26.9-29.5	28.4	27.1-29.7	28.4	27.1-29.8
Nulliparous women (%)	50	38.5-61.5	46	34.2-57.8	47	34.2-59.8
White women (%)	78	68.4-87.6	78	68.2-87.8	70	58.3-81.7
Duration of pregnancy (days)	274	269.6-278.9	273	268.6-277.8	270	265.2-275.4
Oxytocin in first stage (%)	29	18.5-39.5	26	15.7-36.3	20	9.8-30.2
Duration of second stage (minutes)	33	26-40	38	30-47	32	24-40
Oxytocin in third stage (%)	64	52.9-75.1	54	42.2-65.8	66	53.9-78.1
Birth weight (gm)	3309	3143-3475	3276	3122-3432	3087	2904-3270
Placental weight (gm)	508	480-537	501	470-533	510	474-548

*Missing values are maternal age, oxytocin group: 1, control group: 1; nulliparous women, placebo group: 1, control group: 1; duration of second stage, placebo group: 3, control group: 2; birth weight, oxytocin group: 1; placental weight, oxytocin group: 1, placebo group: 2, control group: 2.

was added to the box to equalize the weight of all boxes. However, this ampule was marked "Do not use. Throw away. Control."

Manual removal of the placenta was always performed after induction of general anesthesia. The decision for this surgical intervention was based solely on the clinical judgment of the obstetrician involved.

To investigate possible fetomaternal transfusion associated with umbilical cord injection, two maternal serum samples were taken from a subset of 51 women from groups 1 and 2 for the determination of α-fetoprotein (AFP) levels.[5] The first sample was taken immediately before the intraumbilical injection and the second sample was taken shortly after expulsion of the placenta. Serum AFP concentrations were analyzed with an enzyme-labeled immunoassay (Abbott AFP EIA Monoclonal, Abbott, Amstelveen, The Netherlands).

In all contributing hospitals, total blood loss was noted in a standardized way: during the third stage as much blood as possible was collected in a basin and subsequently measured. Moreover, spill in the sheets was estimated and added to the measured volume.

Duration of pregnancy was based on the last menstrual period if preceded by three normal cycles and if not different by >1 week from a fetal measurement by ultrasonography before 18 weeks, in which case the ultrasonographic measurement was used for its calculation.

Statistical analyses. The results are presented as means, medians, or proportions where appropriate. The dichotomous variable denoted "manual removal of the placenta" was used as the end point of prime interest. To determine whether the population means were all equal, the analysis of variance method was used for continuous variables (maternal age, gestational age, duration of second stage, birth weight, and placental weight).[6] The χ^2 tests were used for dichotomous variables and the Kruskal-Wallis test for variables that were not normally distributed (time interval from injection to spontaneous expulsion of placenta, blood loss). Serum AFP values before and after injection were tested by the Wilcoxon signed-rank test because the interindividual variance of the serum AFP values was very large and not normally distributed. A p value <0.05 was regarded to be significant. Approximation to the 95% confidence interval for proportional values was calculated with the formula described by Armitage[7]:

$$P \pm 1.96 \sqrt{(pq/n)}$$

Results

From the total sample of 220 women who were recruited for the trial, 20 women (9.1%) were excluded from the analyses because of violations of the treatment protocol. Reasons for exclusion were delivery at a pregnancy duration of <28 weeks ($n = 2$), the possible erroneous administration of saline solution from the ampule instead of the requested nontreatment in group 3 ($n = 10$), untimely injection in groups 1 or 2 ($n = 6$), and questionnaires that did not mention the time and way of expulsion of the placenta ($n = 2$). Hence, the final sample comprised 200 women with retained placenta, 72 of whom where randomized to oxytocin treatment (group 1), 69 to placebo treatment (group 2), and 59 to nontreatment (group 3). More than 75% of the women were white, about 10% were of African descent, and the remainder were of mixed Asian (5%) or Mediterranean (7%) origin. No statistical differences existed among these and other characteristics of the three groups (Table I).

No differences between the oxytocin group and the

Table II. Outcome of third stage

	*Oxytocin (n = 72)**		*Placebo (n = 69)**		*Control (N = 59)**	
	Median, proportion	*95% CI*	*Median, proportion*	*95% CI*	*Median, proportion*	*95% CI*
Time interval from injection to spontanous expulsion of placenta (min)†	15‡	—	18§	—	—	—
Manual removal of placenta (%)	38	26.8-49.2	36	24.7-47.3	32	20.1-43.9
Blood loss (ml)	800	—	800	—	650	—

*Missing values are blood loss, oxytocin group: 4, placebo group: 10, control group: 2.
†$p = 0.036$.
‡$n = 39$.
§$n = 37$.

placebo group could be found in the proportion of placentas expelled spontaneously before injection (8% and 10%, respectively) or after injection (both 54%), the injection being given in both groups at a median interval of 45 minutes after delivery of the infant. However, a small but significant difference between both groups was found in the median time interval from injection to spontaneous expulsion of the placenta (Table II). The frequencies of manual removal of the placenta, the major end point of the study, did not differ significantly among all groups and neither did the median volume of blood loss. In practice with the final groups, after exclusion, the power to detect a 30% reduction in the rate of manual placenta removal between the oxytocin and placebo group with $\alpha < 0.05$ was 80%.

In 51 women (25 of group 1 and 26 of group 2) blood samples taken both before injection and after expulsion of the placenta were assayed for AFP. Serum AFP levels did not increase as a result of the intraumbilical injection, but on the contrary a slight decrease was found when each first sample was compared with the second one (medians, 76 and 73.5 μg/ml for the first and second samples, respectively; $p < 0.001$).

Comment

Our study did not show a beneficial effect of the intraumbilical administration of 10 IU oxytocin in 20 ml of saline solution in women with retained placenta apart from a minor reduction in time between injection and spontaneous expulsion of the placenta when oxytocin was given rather than saline solution only. On the contrary, blood loss appeared lowest, although not significantly, in the nontreated control group as compared with the oxytocin-treated group. Because it appeared from the answers to the questionnaire that in the nontreated group an ampule that contained saline solution was injected instead of thrown away, 10 women in this group were excluded from the study. However, six of these 10 women eventually had to have manual placenta removal. When these women were left in the study, the median amount of blood loss in the control group amounted to 800 ml. When the women were added to the placebo group the median amount of blood loss in this group rose to 825 ml. However, in both options no significant difference in the rate of manual placenta removal or in the amount of blood loss among the three groups was obtained.

In fact, the findings of this study are made within the context of obstetric care of the contributing Dutch hospitals and therefore caution should be exercised to extrapolate the results to other populations groups. For instance, the mean age of the study population was >28 years, which is probably high as compared with many other countries. However, the reason for this is not only that more and more Dutch women start a family after completion of their education, but also that manual removal of the placenta more often occurs when age increases. In the period January 1988 to October 1990 in the Academic Medical Centre, the mean age of all women with placenta retention ($n = 292$) was 30.9 ± 0.3 SEM years and without placenta retention ($n = 4959$) 27.8 ± 0.1 years.

Apart from Golan et al.[3] in 1983, several others studied the effect of intraumbilical vein administration of oxytocin.[8-14] To examine the efficacy of this management for retained placenta a randomized placebo-controlled study design is imperative. To date we are aware of only two Danish studies[11, 14] and one English study[15] that made use of this approach. In the first study,[11] a single blind study that involved 51 women randomly allocated to the three treatment groups, no effect of intraumbilical oxytocin (10 IU diluted in 10 ml of saline solution) could be demonstrated on the rate of manual removal of a retained placenta. In the second study, Wilken-Jensen et al.[14] described their

findings of a randomized controlled trial that involved 37 women with a retained placenta. Women allocated to the treatment group (n = 18) were given 100 IU of oxytocin (10 times the dose used by Golan et al.[3] and us) into the umbilical vein. Five of 18 women (28%) in the oxytocin group and 11 of 19 women (58%) who received normal saline solution had to have manual removal of the placenta (not significant). The study by Selinger et al.,[15] which comprised 30 women, did not show an effect of oxytocin.

To determine whether intraumbilical injection of oxytocin shortened the third stage of labor Chestnut and Wilcox[12] and Young et al.[13] conducted a controlled trial in which 10 IU oxytocin in 20 ml of saline solution was administered immediately after delivery of the child. They could not show any effect of the therapy. The small sample size in all these studies could have obscured an effect on the rate of manual removal of the placenta by the intraumbilical injection of oxytocin.

The clinically irrelevant decrease in serum AFP levels in individual women in the interval from the time of injection to after the expulsion or manual removal of the placenta can only be explained by the decrease normally seen after parturition.[16]

In conclusion, this is the first study of which we are aware on women with a retained placenta that evaluates the efficacy of intraumbilical vein administration of oxytocin by the method described by Golan et al.[3] with a randomized, double-blind, placebo-controlled trial design. The benefit of the method appears to be very doubtful because no effect of the intraumbilical vein administration of oxytocin on the relative frequency of manual removal of the placenta or on the amount of blood loss could be demonstrated.

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The role of the transcerebellar view in the detection of fetal central nervous system anomaly

Lyndon M. Hill, MD, Jerry G. Martin, MD, Joanne Fries, RDMS, and Joyce Hixson, RDMS

Pittsburgh, Pennsylvania

The current resolution of antenatal ultrasonography permits the sonologist to visualize and critically evaluate the cerebellum and cisterna magna. Abnormalities in the size and shape of these structures will assist the sonologist in the detection of the majority of central nervous system malformations. (Am J Obstet Gynecol 1991;164:1220-4.)

Key words: Cerebellum, central nervous system anomalies

In 1974, ADR Ultrasound introduced the first commercial real-time B-scan linear array system. With improvements in resolution, finer anatomic detail can be appreciated while at the same time reducing the overall time required for a thorough fetal survey. By 1980, it was estimated that one third of all pregnant women in the United States had had an ultrasonographic examination before delivery. Currently in some countries, 90% to 100% of obstetric patients will have their pregnancy evaluated ultrasonographically. The complex issues of availability, physician training and expertise, and cost are beyond the scope of this article. We would, however, like to address the component parts of an acceptable obstetric ultrasonographic examination of fetal intracranial anatomy. A review of the literature as well as our experience with the transcerebellar view leads us to propose that the cerebellum and cisterna magna should be documented in every fetal anatomic survey after 15 weeks' gestation.

Embryologic development

The cerebellum is derived from neuroblasts on either side of the rhombencephalon beneath the fourth ventricle. By 8 weeks' gestation, cells from the two sides begin to fuse in the midline. The characteristic shape of the cerebellum is a result of the differential growth of its hemispheres in contrast to the median vermis.[1] Ultrasonographically, the transverse cerebellar diameter is consistently visualized after 15 weeks' gestation.

From the Department of Ultrasound, Magee-Womens Hospital, University of Pittsburgh.
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Reprint requests: Lyndon M. Hill, MD, Magee-Womens Hospital, Department of Ultrasound, Forbes Ave. and Halket St., Pittsburgh, PA 15213.
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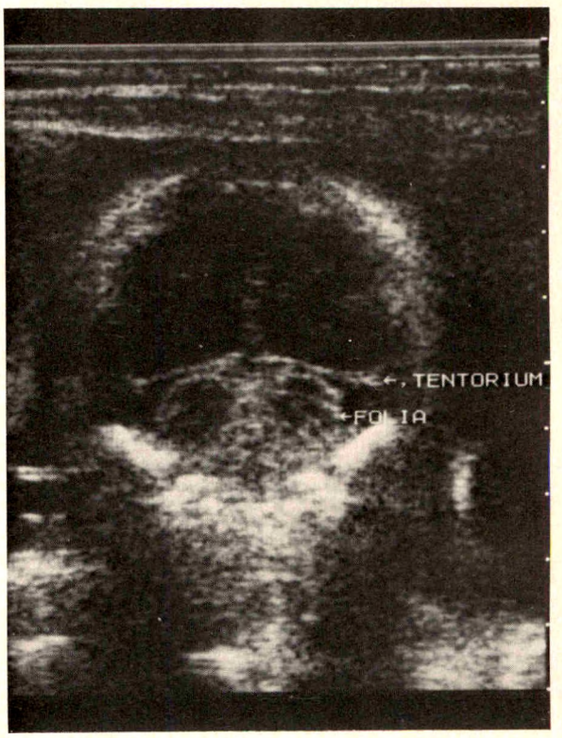


Fig. 1. Coronal view of the cerebellum (26 weeks' gestation). Folia of the cerebellar hemispheres and the tentorium can be visualized.

The cerebellar folial patterns can be appreciated with high resolution real-time scanning (Fig. 1).

Cerebellar function

In general, the cerebellum coordinates both reflex and voluntary motor function. Because the cerebellar hemispheres are phylogenically younger than the mid-

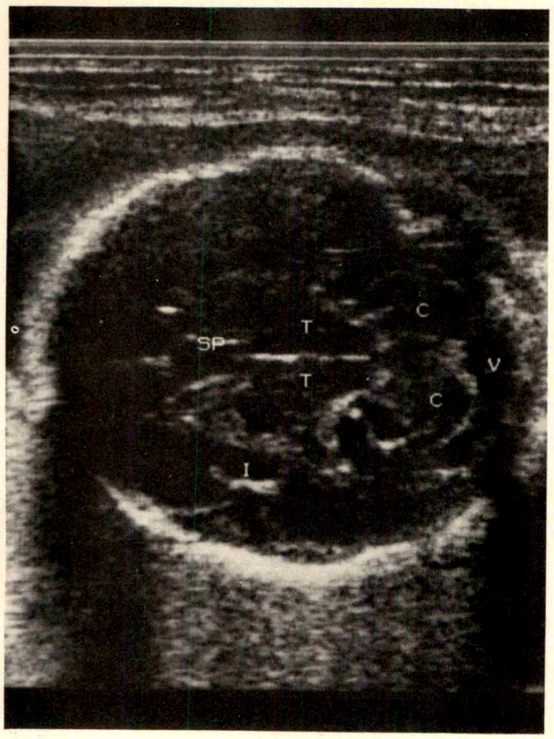


Fig. 2. Axial view of the cerebellar hemispheres *(c)*. The septum pellucidum *(sp)*, thalami *(t)*, insula *(i)*, and echogenic vermis *(v)* within the cisterna magna are also visualized at this level.

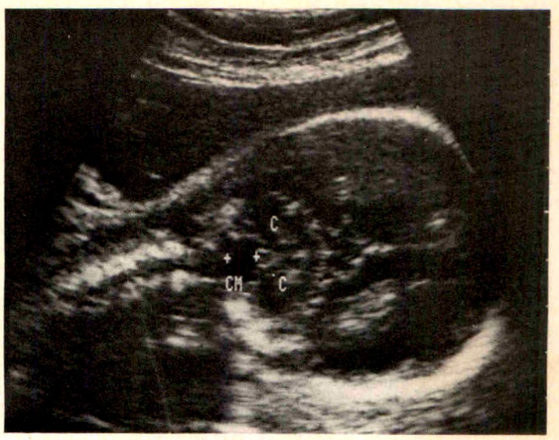


Fig. 3. Graticules indicate the cisterna magna *(cm)* immediately behind the cerebellar vermis (*c*, cerebellar hemispheres).



Fig. 4. *Arrow* demarcates the occipital sinus.

line vermis, the types of motor activity controlled by these areas are different. The cerebellar hemispheres have a primary role in posture and gait, whereas the vermis governs ocular movements. Both areas of the cerebellum contribute to memory, motivational behavior, and learning.[1]

Anatomic-ultrasonographic correlation

The cephalic portion of the cerebellum is immediately posterior to the thalami. The folia of the anterior and quadrangular lobule can also be appreciated on this view. The cerebellar hemispheres, vermis, and cisterna magna can be documented with a slightly greater posterior angulation of the transducer (Fig. 2). An infolding of the dura mater, the tentorium cerebelli (Fig. 1), separates the cerebellum from the cerebrum.

The cisterna magna (Fig. 3) is part of the subarachnoid space that results from the arachnoid bridging between the medulla oblongata and the caudal aspect of the cerebellum. The depth of the cisterna magna is 5 ± 3 mm after 15 weeks' gestation.[2] Occasionally a tubular structure (Fig. 4) is visualized within the cisterna magna. This has been labeled the straight sinus by Mahony et al.[2] and the torcular Herophili (confluence of the sinuses) by Pilu et al.[3] Anatomically, the straight sinus and the torcular Herophili are located at the junction of the falx cerebri with the tentorium cerebelli (i.e., above the plane of the cerebellar hemispheres). Hence the structure visualized within the cisterna magna is most likely the occipital sinus.

Congenital anomalies

Central nervous system anomalies are among the most frequent anomalies encountered by the sonologist. Whereas the diagnosis of anencephaly is not technically difficult, spina bifida as well as infratentorial anomalies are often harder to appreciate.

Nicolaides et al.[4] were the first to emphasize the importance of the intracranial abnormalities associated

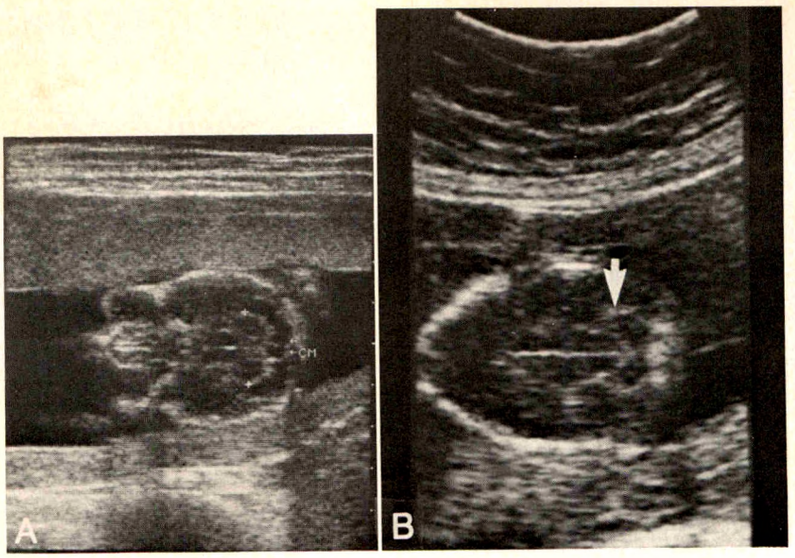


Fig. 5. Axial view of the cerebellum at 18 weeks' gestation. **A,** Normal cerebellum (between the graticules) and cisterna magna *(cm)*. **B,** Banana-shaped cerebellum *(arrow)* and the absence of the cisterna magna in a fetus with a neural tube defect.

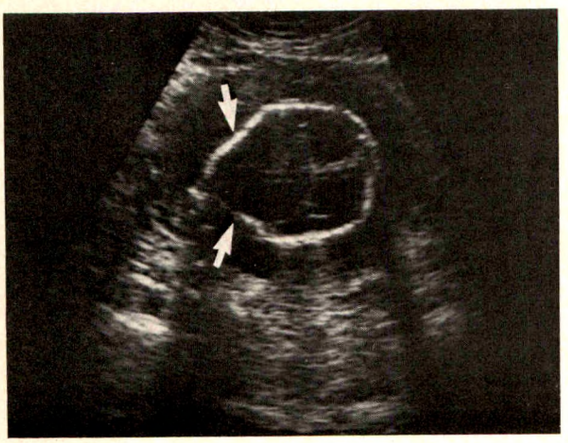


Fig. 6. Axial view of the skull in a fetus at 20 weeks' gestation with a neural tube defect. Scalloping of the frontal bones (lemon sign, *arrows*) is present.

with spina bifida. The cerebellum, in the majority of instances, is deviated into the cisterna magna (banana sign). Consequently, the cisterna magna is virtually obliterated (Fig. 5, *A* and *B*).[5] Scalloping of the frontal bones (lemon sign) (Fig. 6) is a second cranial sign associated with spinal bifida.[4] Finally, the transverse cerebellar diameter in fetuses with spinal bifida is characteristically ≥2 SD below the mean for gestational age.[6] It must be emphasized that the presence or absence of a banana-shaped cerebellum, effacement of the cisterna magna, or lemon-shaped calvarium should not be considered a substitute for a careful evaluation of the fetal spine.

Cerebellar abnormalities are frequently associated with chromosomal anomalies. In the infant with Down syndrome, total brain weight is reduced by 24% and cerebellar weight is decreased by 34%.[7] Therefore we evaluated the transcerebellar diameter in 23 fetuses with Down syndrome between 15 and 20 weeks' gestation to determine whether this measurement could be used as ultrasonographic marker for this particular chromosomal abnormality. The transcerebellar diameter was within the normal range for the cases that were evaluated. These results indicate that either the transcerebellar diameter is insufficiently sensitive to detect the reduction in cerebellar weight or that with Down syndrome the processes that result in cerebellar hypoplasia occur after the second trimester.

Thurmond et al.[8] have reported five cases of trisomy 18 with an enlarged cisterna magna. The transverse cerebellar diameter in these cases were all >2 SD below the mean. Nakamura et al.[9] also detected cerebellar hypoplasia (Fig. 7) in all nine cases of trisomy 18 evaluated between 33 weeks' gestation and 6 months of age. In a retrospective study of 19 trisomy 18 fetuses at our institution, the transcerebellar diameter was ≥2 SD below the mean for gestational age in 11 (57.9%) (unpublished data). The likelihood that the transverse cerebellar diameter would be ≥2 SD below the mean increased with gestational age—two of seven fetuses (28.6%) before 21 weeks' gestation versus nine of 12 (75%) third-trimester fetuses had a cerebellar diameter



Fig. 7. Cerebellar hypoplasia is evident at 35 weeks' gestation in this fetus with trisomy 18.

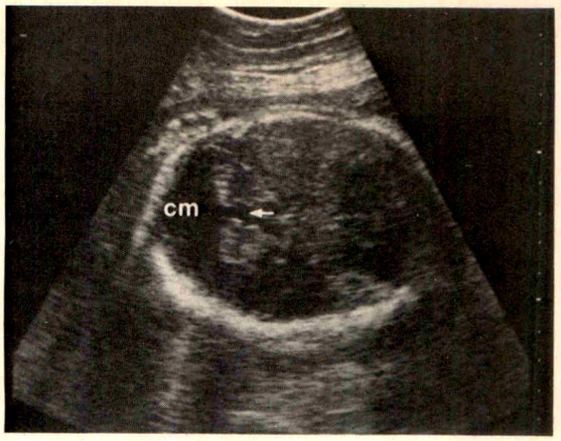


Fig. 8. Dandy-Walker anomaly at 31 weeks' gestation. The cisterna magna *(cm)* is enlarged and the vermis is absent. *(Arrow,* Region of vermis.)

>2 SD below the mean. Two of four fetuses with trisomy 18 that were normally grown at the time of the ultrasonographic examination had a normal transverse cerebellar diameter and two had cerebellar hypoplasia. In contrast, six of 15 fetuses with trisomy 18 that were affected by intrauterine growth retardation had a normal transverse cerebellar diameter whereas the remaining nine fetuses had a transverse cerebellar diameter ≥2 SD below the mean. Hence either an inherent abnormality in brain growth or generalized growth inhibition may give rise to the increased frequency of cerebellar hypoplasia in fetuses with trisomy 18.

Although cerebellar hypoplasia has also been described with trisomy 13, holoprosencephaly is a much more frequent finding.

Cerebellar hypoplasia may be inherited (autosomal recessive).[10] Other causes of cerebellar hypoplasia include viral infection,[11] exposure to teratogens,[12] in utero hypotensive brain stem necrosis,[13] and endogenous factors that alter the bony structure of the posterior fossa.[14]

Cerebellar agenesis may be complete or incomplete. Whereas complete agenesis is extremely rare, incomplete agenesis may occasionally be present without any detectable signs or symptoms.[15] In 1969 Joubert et al.[16] originally described five children with agenesis of the cerebellar vermis associated with ataxia, abnormal eye movements, and mental retardation.

The Dandy-Walker anomaly consists of complete or partial absence of the vermis, a posterior fossa cyst continuous with the fourth ventricle, and in the majority of cases, hydrocephalus (Fig. 8). This malformation is a result of a developmental arrest in the hindbrain that occurs before the third fetal month. Antenatal detection is therefore possible in the latter part of the second trimester. Occasionally the typical ultrasonographic signs of a Dandy-Walker malformation do not become apparent until the third trimester.[17]

An arachnoid cyst will enlarge the cisterna magna, but is not associated with a vermis defect or other cerebellar abnormalities.[2] However, a posterior fossa arachnoid cyst cannot always be distinguished from a Dandy-Walker malformation on antenatal ultrasonography.

It should be remembered that a prominent cisterna magna may be a variant of normal. Similarly, a slightly enlarged or decreased transverse cerebellar diameter may not indicate central nervous system disease, particularly in the third trimester when an accurate measurement can be difficult to obtain.

The American College of Obstetricians and Gynecologists[18] has considered an assessment of the lateral ventricles as well as a transcerebellar view as part of a midpregnancy anatomic survey. Filly et al.[19] evaluated not only the atrium of the lateral ventricle and the cisterna magna, but also the cavum septum pellucidum, as a means of screening for central nervous system malformations. They found that the atrium of the lateral ventricle was enlarged (>10 mm) in 99 of 112 (88%) fetuses with a central nervous system anom-

aly, and seven of the remaining fetuses had an abnormal cisterna magna (<2 mm or >11 mm); the absence of the cavum septum pellucidum did not improve the 95% sensitivity of the other two observations. Our review, and the results of Filly et al.[19] suggest that by incorporating the transcerebellar view in all second- and third-trimester obstetric ultrasonographic examinations, the detection rate of central nervous system anomalies will be improved.

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CA 125 levels in abruptio placentae

Barry R. Witt, MD, Rachel Miles, MD, Gordon C. Wolf, PhD, MD, George T. Koulianos, MD, and Ian H. Thorneycroft, PhD, MD

New Orleans, Louisiana

The diagnosis of abruptio placentae is frequently difficult despite ultrasonography; additional diagnostic parameters would be useful. Maternal serum CA 125, which is believed to derive from the decidua, is elevated in the first trimester and immediately after delivery when placental separation occurs, possibly because of decidual disruption. Serum CA 125 was measured in 27 patients beyond 20 weeks' gestation who were first seen with vaginal bleeding and in 17 control patients of similar gestational age and labor status. Mean (±SD) CA 125 levels were higher ($p < 0.01$) among patients with abruptio placentae (105.9 ± 115 U/ml) than among those with alternate sources of bleeding (13.7 ± 10 U/ml) or control patients (18.2 ± 11.7 U/ml). Mean (±SD) serum CA 125 levels in seven control patients within 6 hours post partum (194 ± 80.5 U/ml) were higher than those among patients first seen with abruptio placentae ($p < 0.01$). Sensitivity and specificity of CA 125 for abruptio placentae were 70% and 94%, respectively. Our data support a decidual source for CA 125 and may indicate utility of CA 125 as a marker for abruptio placentae. (Am J Obstet Gynecol 1991;164:1225-8.)

Key words: Serum CA 125, abruptio placentae

The incidence of abruptio placentae ranges from 0.49 to 1.5% of all deliveries with a perinatal mortality rate of 25% to 60%.[1] Although severe cases of abruptio placentae are characterized by the classic presentation of vaginal bleeding, abdominal or back pain, uterine tenderness, and signs or symptoms of hemorrhagic shock, the milder and more common forms may be difficult to diagnose with certainty. In general, the diagnosis is clinical; however, adjunct evaluation with ultrasonography to rule out placenta previa and identify large retroplacental hematomas may be helpful. That the diagnosis is primarily a clinical one was confirmed in a prospective study[2] in which only one of 59 cases of abruptio placentae were diagnosed by ultrasonography.

Additional noninvasive parameters to assist in the antepartum diagnosis of abruptio placentae would be extremely useful. Previous studies looked at other biochemical parameters to predict abruptio placentae. Salem et al.[3] described elevated levels of placental protein 5 in the serum of five of 12 women with abruptio placentae that were most striking in severe cases. These findings were not confirmed in an apparently similar clinical group.[4] It was also suggested that pregnancy-associated plasma protein A might be predictive of the occurrence of antepartum hemorrhage,[5] but this was not confirmed in a later study.[6]

CA 125, an antigenic determinant associated with ovarian carcinoma, is elevated in maternal serum with peaks detected in the first trimester[7, 8] and in the immediate postpartum period.[7] The origin of CA 125 in pregnancy is uncertain, although recent reports implicated the maternal decidua.[7, 9-14] The aforementioned CA 125 peaks were hypothesized to result from chorionic villi invasion of the decidual cells during early gestation and mechanical destruction of decidual cells during placental separation after delivery.[7] Such decidual disruption would also explain the elevated levels of CA 125 found in the serum of women 1 hour after second-trimester abortion.[7]

The elevation in levels of serum CA 125 after the separation of the placenta led to the current hypothesis that CA 125 may be elevated in pregnancies complicated by abruptio placentae. This preliminary study was undertaken to confirm that hypothesis and to determine whether CA 125 might be useful in the diagnosis of abruptio placentae.

Material and methods

Twenty-seven patients who came to the labor and delivery unit at Charity Hospital of New Orleans with vaginal bleeding after 20 weeks' gestation were included in the study. An additional 17 control patients who were past 20 weeks' gestation were recruited. On arrival at the labor and delivery unit, Institutional Review Board–approved informed consent was obtained and blood was drawn from an antecubital vein. Seven

From the Department of Obstetrics and Gynecology, Section of Reproductive Endocrinology, Tulane University School of Medicine.
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Reprint requests: Barry R. Witt, MD, Department of Obstetrics and Gynecology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Ullmann Research Center for Health Sciences, Suite 123, Bronx, NY 10461.
6/1/26411

Table I. Demographics of study and control groups

	Study group		
	Abruptio placentae	*Other*	*Control group*
No. of patients	10	17	17
Mean (± SD) maternal age (yr)	25.9 ± 6.1	25 ± 4.8	23.1 ± 3.8
Mean (± SD) gravidity	4.1* ± 3.1	2.6 ± 1.5	2.2 ± 1.1
Mean (± SD) weeks' gestation	33.1 ± 5.7	33 ± 4.3	36.4 ± 5.5
No. of stillbirths	3*	0	0
Percentage in labor (No. in labor)	70 (7/10)	53 (9/17)	65 (11/17)

*$p < 0.05$, Abruptio placentae versus other and control group.

Table II. Serum CA 125 levels in study and control groups

Group	*No.*	*Mean ± SD CA 125 (U/ml)*
Study		
Abruptio placentae	10	105.9 ± 115*
Placenta previa	6	9.8 ± 4.9
Other bleeders	11	15.7 ± 11.7
Control	17	18.2 ± 11.7
Postpartum control	7	194.0 ± 80.5†

*$p < 0.01$, Abruptio placentae versus other study patients and abruptio placentae versus controls.

†$p < 0.01$, Postpartum controls versus abruptio placentae.

of the control patients also had blood samples taken within 6 hours post partum. The serum was separated and was frozen at −20° C until assayed. All specimens were obtained between July and December 1989. All assays were run in duplicate and simultaneously to obviate interassay variability.

The CA 125 assay (Centocor, Inc., Malvern, Pa.) used antibody-coated beads and iodine 125–labeled antibody in a solid phase "sandwich" radioimmunoassay. The reference standard was that maintained by the manufacturer. The sensitivity at the lower range of the standard curve was increased by the use of additional dilutions of the standards as described by Pittaway.[15] The intraassay and interassay variations were 5.4% and 7.2%, respectively.

Clinical diagnosis of abruptio placentae was made according to the criteria outlined by Hurd et al.[2] In every case, diagnosis of placenta previa was excluded by ultrasonography, and at the same time, evidence of retroplacental or retrochorionic hematoma was sought. The diagnosis of abruptio placentae was confirmed at delivery in each case with either gross examination of the placenta revealing a firmly adherent retroplacental clot with disruption of the underlying tissue, pathologic diagnosis, or both.

Statistical analysis was performed with analysis of variance, Newman-Keuls tests, and χ^2 analysis. Sensitivity and specificity were calculated with the standard CA 125 cutoff level used for ovarian cancer (>35 U/ml).[16]

Results

The demographics of the study and control groups are summarized in Table I. There are no significant differences between the three groups with regard to maternal and gestational ages or labor status. Gravidity was significantly greater in the group with abruptio placentae, which reflects the higher incidence of abruptio placentae in multigravid women. There were three stillbirths in the study group, all of which were among the patients with abruptio placentae. Despite the differences in gravidity and number of stillbirths, the groups studied are comparable with regard to gestational age, maternal age, and number in labor.

Table II summarizes the serum CA 125 levels of the two groups and of the subdivisions of the study group. Of note are the significantly higher levels of CA 125 in the patients with abruptio placentae ($p < 0.01$) than in the control patients or in patients with other causes of bleeding. The patients with placenta previa tended to have lower levels of serum CA 125 than those of the control group (not significant). Among the patients with other causes of bleeding, one patient had cervical condylomas, one had cervical incompetence, two had low-lying placentas, and the remaining seven had indeterminate causes. In three patients with fetal death, serum CA 125 levels were 23.1, 97.5, and 283.9 U/ml.

There was no correlation between gestational age and serum CA 125 ($r = 0.13$). In addition, there was no difference in mean (±SD) CA 125 levels between all patients in labor (48.6 ± 81 U/ml) and patients not in labor (16.9 ± 13 U/ml).

The mean CA 125 level in the seven control patients who had serum sampled within 6 hours of delivery was significantly higher than the first CA 125 levels measured in the patients with abruptio placentae ($p < 0.01$).

Fig. 1 shows the individual CA 125 levels for each patient.

The sensitivity and specificity for CA 125 in the diagnosis of abruptio placentae were 70% and 94%, respectively.

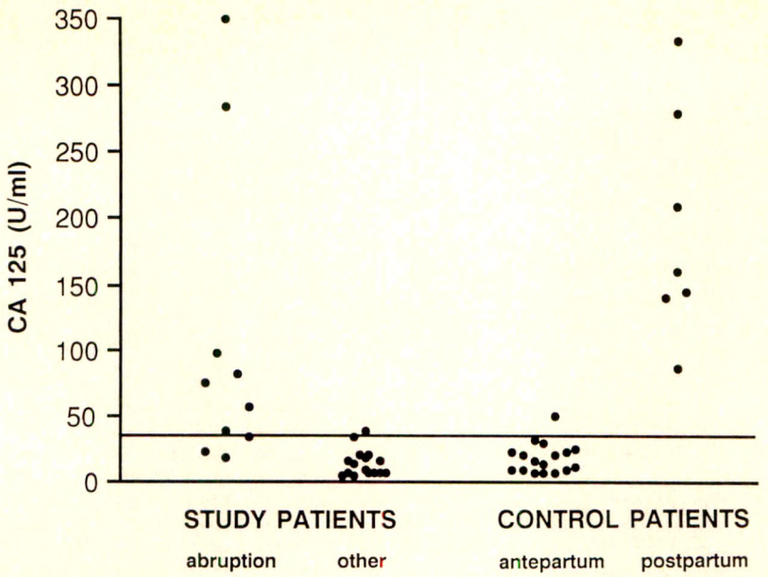


Fig. 1. Individual serum CA 125 levels (units per milliliter) in study patients (abruptio placentae and other causes of bleeding) and control patients (antepartum and post partum). Horizontal line represents serum CA 125 cutoff of 35 U/ml.

Comment

The origin of CA 125 in pregnancy is unclear. The CA 125 antigenic determinant is associated with a glycoprotein with a high molecular weight that is expressed in coelomic epithelium during embryonic development, including müllerian epithelium and the lining of the peritoneum, pleura, and pericardium.[17] Thus the developing fetus was implicated as the source of CA 125 in pregnancy. However, the absence or low level of the antigen in fetal urine and cord blood make this unlikely.[9, 11]

CA 125 was found in high levels in amniotic fluid[9, 11, 18] and it was proposed that CA 125 in the amniotic fluid is produced by the amnion,[11, 13] chorion,[9] or decidua.[12, 13]

There are several reports that support the decidua as the major source CA 125 in maternal serum. Thus high tissue levels of CA 125 were found in first-trimester decidual homogenates[7, 14] and extracts from term decidua.[9] With the use of immunohistochemical staining, CA 125 was localized to the cytoplasm of decidual cells and amnion epithelial cells, but not to the chorion and placental tissue.[13] However, in another study CA 125 was detected in all epithelial cells of the placenta and extraplacental amniotic membranes, both in early gestation and at term.[11] First-trimester decidual cultures[10] and cell cultures from term amnion tissue[19] were shown to produce CA 125 in vitro. It was suggested that although the amnion cells contribute to the high levels of CA 125 in amniotic fluid, the maternal serum CA 125 is derived from decidual production.[7, 11, 14]

The potential of CA 125 as an indicator of abruptio placentae versus other causes of vaginal bleeding in the latter half of pregnancy is supported by review of the cause of these disorders. Abruptio placentae is initiated by hemorrhage into the basal decidua. The decidua then splits, leaving a thin layer adherent to the myometrium.[20] In comparison, one factor in the development of placenta previa is said to be defective vascularization of the decidua.[20] Therefore, it seems reasonable that abruptio placentae with concomitant decidual disruption leads to elevated levels of CA 125, whereas placenta previa, which involves poorly vascularized decidua, might be expected to result in lower levels of CA 125. Our data support this hypothesis and our finding of elevated postpartum CA 125 levels confirm the previous work of Kobayashi.[7] The finding of higher levels of CA 125 in normal postpartum patients than in patients with antepartum abruptio placentae supports the concept of decidual disruption as a cause of CA 125 release in that there are higher levels after complete separation of the placenta (i.e., at delivery) than after partial separation of the placenta (in abruptio placentae).

Previous reports[21, 22] described elevations of CA 125 in cases of intrauterine fetal death but previous studies looked primarily at gestations before 20 weeks. In our study all patients were past 20 weeks' gestation, with a mean (±SD) serum CA 125 level of 134.8 ± 109

U/ml among the intrauterine fetal death patients. However, CA 125 was not elevated in all cases of intrauterine fetal death; one of the patients had a CA 125 level of only 23.1 U/ml. It was suggested that in intrauterine fetal death the CA 125 originates in the destroyed decidua and not in the fetus itself.[7]

With the use of a clinical grading system for abruptio placentae outlined by Sher,[23] with mildest abruptio placentae graded 1 and most severe abruptio placentae graded 3, we classified zero, seven, and three patients as grade 1, 2, and 3, respectively. The mean (±SD) CA 125 levels in patients with grades 2 and 3 were 93.6 ± 115 U/ml and 134.8 ± 134 U/ml, respectively. Thus we were unable to confirm a significant correlation between severity of abruptio placentae and level of CA 125.

The current results suggest that maternal serum CA 125 may have clinical use in the diagnosis of abruptio placentae. Excellent specificity and encouraging sensitivity are noted. Furthermore, CA 125 may have use in the expectant management of gravid women with abruptio placentae, particularly those with significant prematurity.[1] Because the number of patients studied thus far is small, these data must be considered preliminary and additional study is required to corroborate these findings.

In summary, among 27 patients first seen with vaginal bleeding after 20 weeks' gestation, the mean serum CA 125 level among the 10 patients in whom abruptio placentae was diagnosed was significantly higher than those in the other study patients or the control patients. Our findings are compatible with a decidual source for CA 125. These initial findings warrant further study of the utility of CA 125 as a diagnostic test for abruptio placentae or as a marker for following up premature patients being managed expectantly with mild abruptio placentae.

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Management of severe preeclampsia detected in early labor by prostaglandin A_1 or dihydralazine infusions

Mokhtar K. Toppozada, MD, Emad Darwish, MD, and Azza A.T. Barakat, MS
Alexandria, Egypt

The presence of severe pregnancy-induced hypertension at the onset of labor requires therapy with a potent hypotensive agent. Prostaglandin A_1 is a powerful vasodepressor that augments renal blood flow and glomerular filtration and possesses antiplatelet aggregator and oxytocic properties. A continuous intravenous infusion of prostaglandin A_1 (40 to 50 μg/min) or dihydralazine (35 to 50 μg/min) was administered to 20 women with severe preeclampsia (10 in each group). The induced hypotensive response was similar with both drugs but the maximum reduction in blood pressure was achieved sooner with dihydralazine (4 hours) compared with prostaglandin A_1 (7.5 hours). The more gradual hypotensive response is probably less dangerous on placental perfusion than a sudden change. Moreover, the oxytocic property of prostaglandin A_1 shortened the time to delivery, which constitutes another potential advantage. (Am J Obstet Gynecol 1991;164:1229-32.)

Key words: Management of severe preeclampsia, labor, prostaglandin A_1, hydralazine, hypotensive agents

Hypertensive disorders in pregnancy are relatively common complications of gestation and form one side of the great triad of complications (hemorrhage, hypertension, and sepsis) that are responsible for the majority of maternal deaths. Pregnancy-induced hypertension occurs in about 5% to 15% of all pregnancies delivered in large obstetric centers.[1]

Over the past 10 years several studies from the University of Alexandria attempted to evaluate the effects of prostaglandin A_1 (PGA_1) infusions in severe cases of pregnancy-induced hypertension. The results of the investigations were encouraging in that a hypotensive response was elicited and renal functions were markedly improved.[2,3] Moreover, this compound induced a mild oxytocic response and was capable of inducing labor. Also, the weak antiplatelet aggregator property of this agent offered an additional advantage.[4] This beneficial group of responses (renal, cardiovascular, and uterine) appeared to offer a promising potential in clinical practice among subjects not in labor and pregnant >35 weeks.[2-7]

Hydralazine hydrochloride is a well-known, effective antihypertensive agent. Most authorities concur that it is the agent of choice for the treatment of severe pregnancy-induced hypertension. When hydralazine was used in human beings a fall in blood pressure and in total peripheral resistance was found inasmuch as it exerts a direct vasodilator effect on peripheral arterioles. It improved uterine circulation and has a beneficial effect on cerebral, retinal, and renal circulation in pregnancy.[8]

The aim of this work was to evaluate the clinical use of PGA_1 as an antihypertensive agent and as a weak oxytocic agent in patients with severe preeclampsia who were in early labor. The clinical performance of this drug was compared with that achieved with another hypotensive drug, hydralazine, in a similar group of patients.

Patients and methods

This study comprised 20 pregnant patients with gestational ages that ranged from 28 to 40 weeks. All patients had the manifestations of severe preeclampsia (i.e., with a systolic blood pressure ≥160 mm Hg or a diastolic blood pressure ≥110 mm Hg at bed rest). Edema and proteinuria were present in all patients.

The general condition was evaluated, and whenever possible it was confirmed by history and examination that these patients did not originally have essential hypertension or chronic nephritis. Patients with bronchial asthma, glaucoma, and severe systemic disorders were excluded.

The obstetric examination was within normal limits, with vertex presentation and adequate cephalopelvic relationship. The examination and tocographic findings confirmed that the patients were in early labor by regular, rhythmic uterine contractions (three contractions per 10 minutes) accompanied by effacement and dilatation of the cervix. Moreover, there was formation

From the Department of Obstetrics and Gynaecology, Shatby Hospital, the University of Alexandria.
Received for publication February 23, 1990; revised October 10, 1990; accepted November 9, 1990.
Reprint requests: M.K. Toppozada, MD, Shatby University Hospital, Shatby, Alexandria, Egypt.
6/1/26705

Table I. Mean ± SD of the admission data of the two groups

	Group I	*Group II*	*t*
Age (yr)	29.7 ± 4.4	29.0 ± 4.2	0.16
Gravidity	1.8 ± 1.35	2.3 ± 1.6	0.34
Parity	0.8 ± 1.13*	1.1 ± 1.4†	0.24
Gestational age (wk)	38.85 ± 1.31	38.8 ± 1.8	0.51
Cervical dilatation (cm)	3.79 ± 0.4	3.9 ± 0.5	0.19
Blood pressure (mm Hg)			
Systolic	183.0 ± 11.6	180.9 ± 17.1	0.17
Diastolic	116.0 ± 7.0	120.5 ± 7.6	0.48

t, Student's test. It is not significant in any item ($p > 0.05$)
*6 Primigravida, 4 multigravida.
†5 Primigravida, 5 multigravida.

of the bag of forewaters if the membranes were not ruptured. The cervix was dilated to <5 cm. The case material was divided at random with a computer-generated table into two groups according to the specific therapy that was used.

Group I. Ten patients were given PGA_1 infusions (kindly provided by Prof. Sune Bergstrom, Karolinska Institute, Stockholm, Sweden) in a dose of 40 to 50 μg/min, which is equivalent to the recommended dose of 0.5 μg/kg per minute for women who weigh 80 to 100 kg.

Group II. Ten patients (controls) were given dihydralazine (Nepresol antihypertensive agent, Ciba-Geigy, Basel, Switzerland) infusions in a dose of 35 to 50 μg/min.

Apart from sedation with diazepam, none of the subjects selected for the present investigation received any other medication.

PGA_1 was available in vials; each vial contained 3 ml (10 mg/ml). The infusion was prepared by adding 2 to 2.5 ml of PGA_1 into 500 ml bottles of 5% glucose (40 to 50 μg/ml); infusion pumps were used to provide the precalculated dose of 1 ml/min.

Dihydralazine was available as Nepresol antihypertensive agent in powder form in ampules of 25 mg, plus ampules of 2 ml solvent. The infusion was prepared by adding one ampule of dihydralazine and one ampule of the solvent into 500 ml bottles of 5% glucose (50 μg/ml).

Before and during infusion and until delivery occurred, the following parameters were carefully monitored: (1) blood pressure was continuously monitored with the Dinamap vital sign monitor (Critikon, Inc., Tampa, Fla.); (2) uterine activity and fetal heart rate were also continuously monitored by external methods; (3) clinical course and progress of labor were followed up closely and any side effects or adverse reactions were recorded; (4) obstetric outcome and infusion-delivery period were assessed, measurement of blood pressure was continued for 48 hours after delivery, and clinical assessment of the neonates with Apgar scores at 1 and 5 minutes was performed.

Results

There were no significant statistical differences between the two groups with regard to the mean age, gravidity, parity, and gestational age. There were no differences in the level of systolic and diastolic blood pressures of the two groups or in the degree of cervical dilatation on admission (Table I).

Changes in blood pressure in the two groups. After the onset of the PGA_1 infusion in group I the systolic and diastolic blood pressure levels decreased gradually in all patients. The mean of the lowest recorded readings of systolic blood pressure achieved at various time intervals in different subjects was 142 ± 11.4 mm Hg. There was a highly significant reduction compared with admission values (Student's *t* test, 17.6; $p < 0.01$). The mean of the lowest diastolic pressure recorded during infusion was 92.5 ± 5.9 mm Hg, which was also significantly lower than admission values (Student's *t* test, 11.9; $p < 0.01$). At the onset of the PGA_1 infusion the mean arterial blood pressure (MAP) was 138.3 ± 5.6 mm Hg. During the period of the infusion the mean value of the minimum MAP (achieved at various time intervals) was 109 ± 7.4 mm Hg. The reduction in the MAP was highly significant (Student's *t* test, ±16.5; $p < 0.01$).

After the onset of the dihydralazine infusion in group II, the systolic and diastolic blood pressure levels decreased gradually in all patients. The mean of the lowest recorded readings of systolic and diastolic blood pressures were 130.5 ± 7.6 and 90.5 ± 5.0 mm Hg, respectively. There was a highly statistically significant reduction in systolic and diastolic blood pressure levels during treatment with dihydralazine infusion compared with admission values ($p < 0.01$).

During the period of dihydralazine infusion, the mean value of the minimum MAP achieved at various time intervals was 106.07 ± 9.5 mm Hg. The reduction in the MAP was also highly significant (Student's *t* test, 10.7; $p < 0.01$) (Fig. 1, Table II).

Forty-eight hours after delivery, the mean systolic/diastolic blood pressure levels were 137.0 ± 8.23/91.5 ± 5.30 in group I and 132.0 ± 4.22/90.0 ± 6.67 in group II. These values were statistically highly significant when compared with admission levels ($p < 0.01$) but not different from the minimum values recorded during infusion ($p > 0.05$). The time needed for the blood pressure to reach minimal levels during infusions is presented in Table III, which shows that the lowest recordings were achieved in a shorter time in group II.

Table II. Mean of the maximum reduction in blood pressure during infusions of PGA_1 or dihydralazine in groups I and II

	Group I		*Group II*	
	Maximum reduction	*t**	*Maximum reduction*	*t**
Blood pressure (mm Hg)				
Systolic	41.0 ± 7.38	17.6	50.0 ± 19.6	8.1
Diastolic	23.5 ± 6.26	11.9	31.0 ± 9.3	10.1
MAP (mm Hg)	29.3 ± 5.63	16.5	36.3 ± 10.8	10.7

*Compared with admisssion values, $p < 0.01$, Student's t test.

Table III. Mean time needed (hours) to reach minimum blood pressure after the onset of infusion in the two groups

Blood pressure	*Group I*	*Group II*	*t**
Systolic	7.6 ± 2.63	4.2 ± 1.32	3.654
Diastolic	7.5 ± 1.35	3.9 ± 1.26	6.164

*Significant, $p < 0.05$, Student's t test.

Changes in pulse rate. There was a significant increase in pulse rate in the two groups during infusion. The maximum values were achieved at various time intervals during infusion. In group I, the mean initial pulse rate (i.e., before starting the infusions) was 79.0 ± 6.1 beats/min, whereas the mean of the maximum pulse rate during infusion was 94.4 ± 5.9 beats/min (Student's t test, 7.9; $p < 0.01$). In group II the mean initial pulse rate was 79.8 ± 8.5 beats/min, whereas the mean of the maximum pulse rate was 84.4 ± 10.1 beats/min (Student's t test, 4.4; $p < 0.01$). By comparing the mean pulse rate of the two groups it was observed that there was a significant difference between group I and II (pooled Student's t test, 7.17; $p < 0.01$).

Course of delivery. In group I, the mean of the infusion delivery interval was 7.7 ± 3.1 hours, whereas in group II it was 10.12 ± 2.5 hours; the difference between both groups was significant (pooled Student's t test, 1.9; $p < 0.05$). The duration of labor among women who had vaginal deliveries in both groups (nine in group I and eight in group II, i.e., excluding women who had cesarean deliveries) were 7.49 ± 3.21 and 10.10 ± 1.34 hours, respectively (Student's t test, 2.13, $p < 0.05$).

In group I, eight patients were delivered spontaneously during PGA_1 infusions and 1 patient was delivered by vacuum extraction. A cesarean section was performed because of cervical dystocia and premature rupture of membranes in one patient. In group II, seven patients were delivered of infants spontaneously

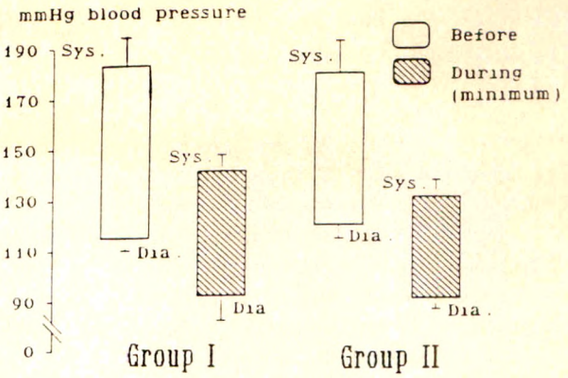


Fig. 1. Mean ± SD of systolic *(Sys.)* and diastolic *(Dia.)* blood pressure (mm Hg) in both groups before onset of infusions and mean of minimum level recorded during therapy.

during dihydralazine infusions and one patient was delivered by low forceps. Cesarean section was done because of fetal distress in one patient (severe bradycardia) and cephalopelvic disproportion in another patient.

In group I, fetal bradycardia was detected in one patient at various time intervals. The rest of the patients showed normal fetal heart rate patterns. In group II, phases of temporary bradycardia were recorded in three patients at various time intervals during infusion of dihydralazine. One of these patients was delivered by cesarean section because of fetal distress, whereas the others showed improved patterns on position change and oxygen inhalation. The rest of the patients showed normal patterns. There were no serious maternal side effects during infusion in both groups. All recorded side effects were temporary and were spontaneously relieved by decreasing the rate of infusions.

In group I, the mean of the Apgar scores at 1 minute was 9.2 ± 0.9 and 10 at 5 minutes, whereas in group II the corresponding figures were 9.2 ± 0.7 and 10, respectively. This shows that there was no difference in the Apgar scores of the two groups.

Comment

Hypertensive disorders are serious complications of gestation and constitute a major cause of maternal mortality. They also represent an important cause of perinatal mortality and severe morbidity.[6]

For many years the lines of therapy of preeclampsia depended on the control of the manifestations (edema, proteinuria, hypertension) and prevention of complications. In severe preeclampsia and in cases that do not respond to therapy, termination of pregnancy represents a life-saving procedure.

Recently, PGA_1 was clinically tested in the treatment of pregnancy-induced hypertension.[6, 7] The adminis-

tration of PGA_1 by intravenous drip in a dose of 0.5 μg/kg per minute induced a hypotensive response that is probably by means of peripheral arteriolar dilatation. It also augmented the renal blood flow, almost doubled glomerular filtration rate, and showed a weak uterine stimulatory effect in term pregnant women.[3, 5, 6]

It was later found that PGA_1 infusion alone was insufficient to induce labor in all treated patients, especially among subjects with unripe cervices. PGA_1 infusion together with ripening of the cervix with PGE_2 vaginal or cervical gel successfully induced labor within 24 hours in most of the patients.[7]

Dihydralazine group of drugs is known to improve kidney perfusion, may improve placental perfusion, and is effective in the reduction of premature births and still births.[9]

In the present work the aim was to use PGA_1 or dihydralazine to suppress the high blood pressure of severe preeclamptic toxemia to normotensive levels during the progress of spontaneous labor. The dose of PGA_1 infusion used was determined on the basis of previous experience with the same drug in Shatby Hospital.[5, 6] However, a fixed dose range of 40 to 50 μg/min was used in the present work, whereas in previous studies the dose was calculated according to the subject's weight (0.5 μg/kg per minute).

The two groups were comparable with respect to all admission criteria. Patients in both groups showed a good hypotensive response. Also, there was no rebound rise in blood pressure after delivery. This is probably because of the proper selection of the patients without any element of essential hypertension. The more gradual reduction of blood pressure in group I is less likely to decrease the uteroplacental perfusion than the rapid drop in blood pressure observed in group II.

The infusion-delivery interval was calculated from the time of starting the intravenous infusions, and it was significantly longer in group II with the use of dihydralazine than that in group I with PGA_1. This difference is an obvious advantage and is in agreement with the observation that PGA_1 is a uterine stimulant and has the ability to induce labor in term pregnant women.[2, 6, 7, 10]

All neonates in the two groups had good Apgar scores. The fetal heart rate changes during PGA_1 and dihydralazine infusions were generally temporary, and within the expected range of occurrence in severe cases of preeclampsia or placental insufficiency. These results are reassuring that the treatment used may not increase the fetal risks in such serious cases. However, some studies showed that patients with severe pregnancy-induced hypertension who received hydralazine had a significantly higher incidence of fetal distress than that of those who did not receive the drug.[11] In group I, the cesarean section rate (one of 10 patients) was relatively low and rather similar to previous studies with PGA_1.[5]

Comparison of the two lines of treatment in the present study shows both offered a good hypotensive response with no obvious serious side effects. With PGA_1 infusion, the reduction in blood pressure was more gradual and needed more time to reach its lowest level than that in group II with dihydralazine. Rapid reduction may compromise the fetus by decreasing the uteroplacental perfusion, but it may offer an advantage during the stress of an ongoing labor. PGA_1 infusion offers a shorter mean duration of infusion-delivery interval in view of its uterine stimulating property. This may represent another advantage to both mother and fetus.

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The intrauterine probe electrode

Thomas H. Strong, Jr., MD,[a, b] Richard H. Paul, MD,[a, b] George D. Park,[a, b] Yi Cheng, PhD,[c] Donald E. Lewis,[a, b] Dorothy F. McCart, RN,[a, b] and Evelyn A. Mueller, RN[a, b]

Los Angeles and Pomona, California

An intrauterine probe electrode was inserted into 100 laboring women and 366 bipolar electrode combinations were tested. A noise-canceling technique was used with the final 28 subjects to remove competing maternal cardiac signals. Twenty-four (86%) had fetal heart rate tracings with sufficient technical quality to allow determination of the baseline fetal heart rate. (AM J OBSTET GYNECOL 1991;164:1233-4.)

Key words: Fetal electrocardiogram, fetal heart rate monitoring, intrauterine probe electrode

We developed a device to obtain the fetal electrocardiogram (ECG) for fetal heart rate (FHR) derivation without puncturing fetal skin.

Material and methods

One hundred women in labor at Los Angeles County/University of Southern California Women's Hospital in Los Angeles were selected for insertion of the device. The project was approved by our Institutional Review Board. All patients gave consent before insertion.

Each electrode consisted of 22-gauge, silver-silver chloride–plated, sterling silver wire wrapped along 0.25-inch segments of inert tubing used to measure intrauterine pressure. A 26-gauge copper wire connected each electrode to a standard fetal monitor leg plate. A varying number of electrodes was placed on each probe.

The technique for inserting the device was identical to that for intrauterine pressure catheter placement. The device was gently inserted to a depth of 12 to 18 inches. A fetal scalp electrode was also applied.

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine,[a] Women's Hospital, Los Angeles County/University of Southern California Medical Center,[b] and the Departments of Electrical and Computer Engineering, California State Polytechnic University, Pomona.[c]
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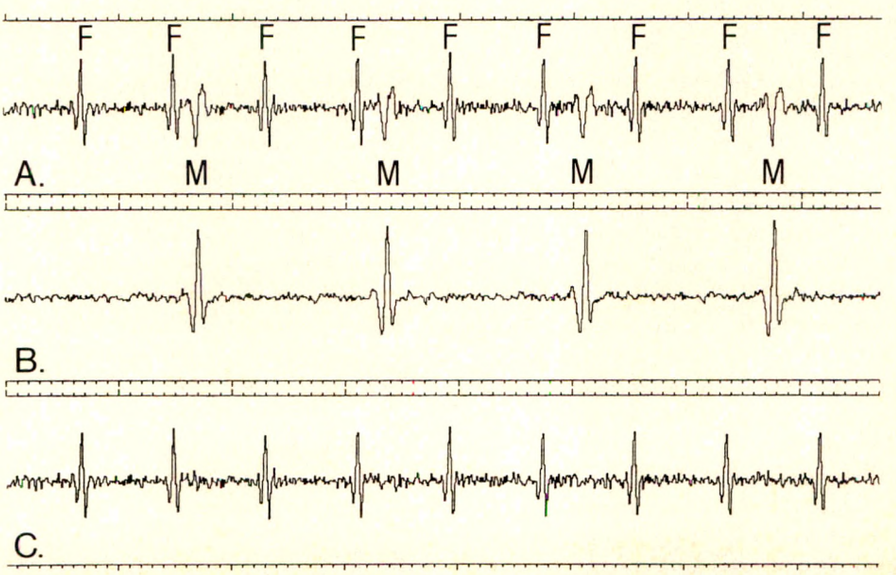


Fig. 1. Electronic processing of the probe-electrode signal. *A*, The probe-electrode signal. *B*, The maternal chest ECG. *C*, The processed signal after electronic elimination of signal *B* from signal *A*.

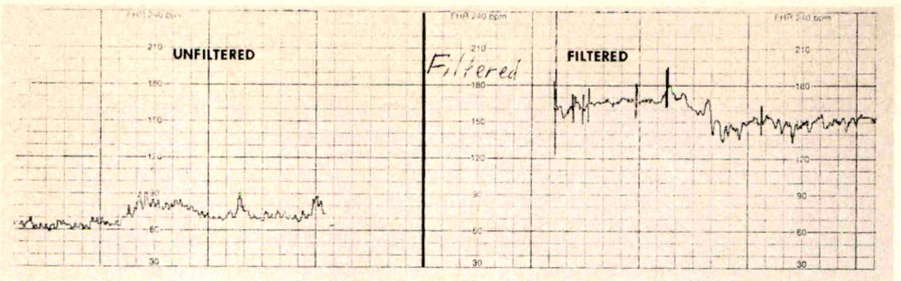


Fig. 2. The effect of electronic signal processing. Baseline heart rate switches from the maternal to the fetal rate.

The amplitude of the fetal QRS complex in microvolts was determined for both the fetal scalp electrode and probe device with a Hewlett-Packard 7826B single-channel ECG rhythm strip recorder. The ECG voltages obtained from the fetal scalp electrode and probe electrode were compared. Then the probe electrode was connected to a portable Corometrics 115 fetal monitor. The FHR tracings derived from the fetal scalp electrode were compared with those concomitantly obtained from the probe electrode.

To remove the competing maternal ECG from the signal, the final 28 devices used fetal ECG signal processing. An adaptive noise-canceling technique similar to that used by Widrow[1] transformed the maternal chest ECG signal to match the maternal ECG components found in the probe-electrode signal. The maternal chest ECG was then subtracted from the probe-electrode signal (Fig. 1). The processed tracings were considered improved if the device switched from a maternal baseline to a FHR baseline. If the probe electrode was tracking the FHR baseline before activating the signal processor, the "after" tracing was judged to be improved if the baseline FHR showed less artifact (Fig. 2).

Results

The mean fetal QRS complex voltage for all 366 bipolar electrode combinations tested was 40 μV and the mean fetal scalp electrode signal was 234 μV. The specific interelectrode distance of 9 inches (n = 92) produced the greatest mean fetal ECG voltage—66 μV (range, 10 to 875 μV). Electronic background noise noted during evaluation of the fetal R wave did not exceed 5 μV (peak to peak method; signal band width used, 15 to 40 cycles/sec).

When the 100 devices were connected to the fetal monitor, 46 produced FHR tracings. However, the majority of these FHR tracings had significant amounts of artifact. Among the final 28, the fetal tracing was improved in 20 (71%) with signal processing. However, among the eight not improved by the signal processor, four were already of good technical quality. Therefore the fetal probe electrode with signal processing had a success rate of 86% (24 of 28) in which the baseline FHR, variability, accelerations, and decelerations were discernible. When compared with tracings concomitantly obtained by fetal scalp electrodes, there was more artifact in the probe-electrode tracings.

Comment

Elimination of the competing maternal ECG enhances the ability to count the fetal ECG detected by the probe-electrode device. Additional electronic processing of the raw fetal ECG will likely further clean up the FHR tracing. However, a careful balance must be sought in this process. A visually pleasing FHR record may not mean that it is precise.

With a similar device, Randall et al.[2] achieved maximal fetal signals with an interelectrode interval of 7 inches. Therefore steric and electronic constraints suggest that the optimal electrode spacing for the device is 7 to 9 inches.

Randall et al.[2] argue that amniotic fluid attenuates the available signals. Therefore we propose that actual electrode contact with the fetal skin may be the critical factor for this type of fetal ECG signal detection.

We have developed and tested a device to detect fetal electrocardiac activity in labor that leaves the fetal skin intact. With further signal processing, this device may become clinically useful for continuous intrapartum FHR monitoring, especially if a single-probe device were equipped for simultaneous FHR and intrauterine pregnancy monitoring.

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Management of radionecrosis of the vulva and distal vagina

William S. Roberts, MD,[a, c] Mitchel S. Hoffman, MD,[a, c] James P. LaPolla, MD,[a, c] Ernesto Ruas, MD,[b, d] James V. Fiorica, MD,[a, c] and Denis Cavanagh, MD[a, c]

Tampa, Florida

Twelve patients were seen between January 1983 and June 1989 with the clinical diagnosis of radionecrosis of the vulva or distal vagina. Seven patients received radiation for vulvar cancer, three for distal vaginal cancer, and two for recurrent endometrial cancer. No patient healed spontaneously and the mean delay in surgical therapy was 8.5 months. The radionecrotic site was treated with local therapy, radical local excision (with or without colostomy), or exenteration. The operative defect was closed primarily in three patients and covered with local flaps or myocutaneous flaps in seven patients. The two patients with local care still have radionecrotic ulcers. One of three patients who were closed primarily continues to have an ulcer. All other patients have healed satisfactorily except one who died after two attempts to correct the problem. Radionecrosis of the vulva and distal vagina should generally be treated surgically. (Am J Obstet Gynecol 1991;164:1235-8.)

Key words: Radionecrosis of the vulva

Delayed radiation injury is an unfortunate fact of life when curative doses of radiation therapy are given. The frequency and severity of these injuries are directly related to the total dose of radiation given to the affected area.[1] The exact mechanism of damage is unclear but the result is progressive fibrosis and loss of blood supply in the radiated tissue. This process may ultimately result in frank necrosis.

The skin was a frequent site of injury when orthovoltage radiation was in use. With the development of megavoltage radiation, injury to the skin has become much less common. Vulvar skin is notoriously less tolerant of radiation therapy than skin covering other areas of the body. Suggested reasons for this include moisture, friction, a nonflat surface, and a larger proportion of end arteries than that in other areas of skin. Despite this, radiation injury of the vulva occurs very infrequently.[2] In large part, this is because vulvar cancer is primarily treated surgically. When radionecrosis does involve the vulva or distal vagina, the typical patient is in severe pain and is hesitant to go out in public because of the foul odor. The patient is generally treated with narcotic analgesic agents, antibiotics, debridement, and intensive local care. A hyperbaric oxygen chamber may also be used if available.[3] Unfortunately, this intensive, conservative therapy is ineffective in most patients. We report our experience with the management of this rare clinical problem.

From the Divisions of Gynecologic Oncology[a] and Plastic Surgery,[b] Departments of Obstetrics and Gynecology[c] and Surgery,[d] H. Lee Moffitt Cancer Center and Research Institute, University of South Florida.
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Material and methods

The charts of all patients with the clinical diagnosis of radionecrosis of the vulva or distal vagina managed by members of the Division of Gynecologic Oncology at the University of South Florida between Jan. 1, 1983, and June 1, 1989, were retrospectively reviewed. Any patient with obvious persistent or recurrent cancer within a necrotic tissue bed was excluded. A total of 12 patients were identified with a mean age of 68 years and a range of 34 to 85 years. Ten patients were white and two were black. The primary tumor site for which the patient received radiation and the International Federation of Gynecology and Obstetrics (FIGO) clinical stage are listed in Table I. All three patients with vaginal cancer had distal vaginal lesions, but both patients with recurrent endometrial cancer had vaginal apical lesions. The methods of radiation administration as well as accompanying or past therapies were extremely variable and are listed in Table II. Previous surgery was radical vulvectomy in two patients. Chemotherapy was concomitant intravenous cisplatin and 5-fluorouracil in one patient, intravenous bleomycin and 5-fluorouracil in one patient, and intraarterial cisplatin and floxuridine in one patient. The patient treated with surgery and external radiation had a wide local excision of the vulvar cancer. All external radiation was administered with a cobalt unit or linear accelerator. Vaginal brachytherapy was delivered with a Burnett cylinder. The maximum total dose of radiation to the radionecrotic site was estimated. The mean dose was 6699 cGy with a range of 4500 to 9400 cGy. Seven patients had some chronic medical conditions that may have contributed to a compromised blood supply in the radiated area and these are listed in Table III. Five patients had no identifiable predisposing conditions.

Table I. Site of cancer and stage

Primary site	*Stage*	*No. patients*
Vulva	I	1
	II	2
	III	1
	IV	1
	Recurrent	2
Vagina	II	3
Endometrium	Recurrent	2
TOTAL		12

Table II. Cancer treatment details

Modality	*No. patients*
Previous surgery, chemotherapy, external radiation, interstitial radiation	3
External radiation, interstitial radiation	2
External radiation, superficial vaginal brachytherapy	3
Surgery, external radiation	1
External radiation only	1
Interstitial radiation only	2
TOTAL	12

Table III. Associated medical conditions

Condition	*No. patients*
Essential hypertension	4
Noninsulin-dependent diabetes	3
Smoking	2
Previous stroke	1
Previous myocardial infarction	1
No predisposing conditions	5

Three patients had more than one condition.

The distribution of these chronic conditions in the study population is similar to that of the general elderly population. The mean time from completion of radiation to clinical evidence of necrosis was 16.2 months with a range of 3 to 69 months. All patients were initially treated with antibiotics, surgical debridement, and intensive local care including hydrogen peroxide, honey, and several other similar agents. One patient with a particularly large ulcer (Fig. 1) was treated intensively with hyperbaric oxygen.

Results

Conservative therapy did not result in even partial healing in any patient. Ten patients were ultimately treated with radical excision of the radionecrotic site. The two patients who were not treated surgically were elderly and not considered to be surgical candidates. Six of the 10 patients underwent radical excision of the radionecrotic site without removal of pelvic viscera. One of these patients had a colostomy because of the proximity of the ulcer to the anus and anal sphincter. This colostomy was later closed. Three patients required pelvic exenteration: one total, one anterior, and one posterior exenteration. One patient was initally treated with radical excision and colostomy but eventually required a total pelvic exenteration. Although it was not clinically suspected, three of the 10 patients had recurrent tumor in the excised specimens. Recurrent cancer developed later at the radionecrotic site in one patient not treated surgically. In the 10 patients treated surgically, the mean time from diagnosis of radionecrosis to surgical therapy was 8.5 months with a range of 2 to 20 months. Responsibility for this delay was shared by both ourselves and referring physicians.

The methods of tissue coverage after excision were variable and are seen in Table IV. The patient who underwent radical local excision before total pelvic exenteration had two separate attempts at coverage so the total in Table IV is 11 rather than 10. Of the 10 patients treated surgically, eight ultimately healed completely. One patient who had radical local excision with primary closure that failed continues to have a necrotic ulcer. Both coverage attempts failed in the patient who underwent radical local excision before pelvic exenteration and she ultimately died of surgery complications. Autopsy revealed no evidence of cancer. The coverage attempts in this particular patient were a unilateral gracilis myocutaneous flap that preceded local buttock flaps. Of the eight patients who healed completely, two had partial flap necrosis that resulted in introital stenosis. One of these patients underwent successful surgery to correct this problem. Three of these eight patients are sexually active. One of the two patients not treated surgically continues to have a radiation ulcer that is unchanged. The other patient died of cancer within the radiation ulcer. Of the 10 patients treated surgically, seven are alive without evidence of disease, two are dead of disease, and one died without evidence of disease as already mentioned. Recurrent problems have not developed in any patient who healed after surgical therapy.

Comment

Surgical treatment of radiation injuries of the skin has been advocated for many years.[1] This is classically composed of radical excision of all radiation-damaged tissue before appropriate tissue coverage. Whether this is widely practiced is unknown. Bloomer and Hellman[5] state, "These problems demand close cooperation between experienced surgeons and radiotherapists. Be-

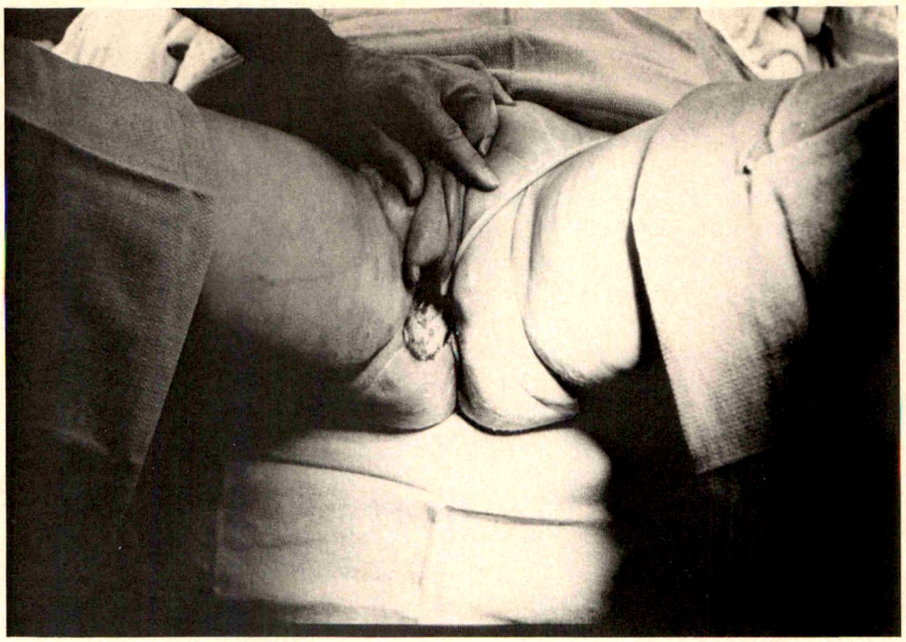


Fig. 1. Large radionecrotic ulcer of the vulva.

cause of the limited blood supply in the involved areas, conservative treatment is often all that is recommended." In our experience conservative therapy does not produce satisfactory results in the great majority of patients with radionecrosis of the vulva. Limited blood supply in the involved areas is precisely why conservative therapy does not result in complete healing.

Radionecrosis of the vulva is clearly a rare occurrence. Radiation to the vulva is seldom used in the primary therapy for carcinoma of the vulva. In the era of orthovoltage radiation necrosis developed in 30% of patients radiated for carcinoma of the vulva.[6] Megavoltage γ-radiation with or without interstitial therapy results in a 1.8% to 5% incidence of vulvar necrosis.[2, 7] With the use of electrons alone, Frischbier[8] reported a 4.5% incidence of radionecrosis of the vulva. In our experience interstitial radiation with or without external radiation carries a 60% risk of necrosis.[9] In recent years there has been increasing interest in radiation therapy combined with limited surgery in patients with advanced vulvar cancer.[10] The incidence of radionecrosis in these series was minimal but as interest in this approach increases the problem will likely become more common. There has also been interest in combining chemotherapy with radiation therapy in patients with advanced vulvar cancer.[11] Whether chemotherapy enhances the effect of radiation on normal vulvar skin remains to be seen. This approach may increase the incidence of radionecrosis of the vulva. Three patients in our series had chemotherapy combined with radiation therapy.

Table IV. Reconstruction after excision of radionecrotic site

Primary closure	3
Unilateral gracilis myocutaneous flap	3
Unilateral gracilis muscle flap covered with split-thickness skin graft	1
Bilateral gracilis myocutaneous flaps	2
Bilateral femoral fasciocutaneous flaps	1
Bilateral local buttock flaps	1
TOTAL	11

Late radiation damage is related to the total dose received, the dose rate, and the volume of the tissue radiated. In this series the total dose was variable and as little as 4500 rad was associated with necrosis. In two patients with recurrent endometrial cancer at the vaginal apex it was not intended to radiate the vulva at all. It can only be assumed that the Burnett cylinders were displaced in both patients. It is obvious that radionecrosis can occur even with relatively small doses of radiation.

In our experience conservative management did not result in healing in any patients with vulvar radionecrosis despite many months of trying. In our opinion if healing has not occurred in 1 to 2 months, surgery should be considered. With radical excision of all radiation-damaged tissue before appropriate tissue coverage, eight of 10 patients healed completely with good quality of life. Unfortunately, four of 10 patients required pelvic exenteration to remove all the radiation-damaged tissue. Two patients required a

colostomy and in our opinion one of these patients had preservation of the anus and fecal continence as a result of the temporary colostomy.

Tissue coverage was accomplished with primary closure in three patients. This resulted in complete healing in two patients. Primary closure requires a small defect with mobile surrounding tissues. Closure without tension is critical in this situation. Primary closure will not be appropriate in the majority of patients with this problem. Seven patients were closed with flaps, six of which were gracilis flaps. In one of these patients the gracilis muscle alone was used to fill the defect and was covered by a split-thickness skin graft. In this particular patient the gracilis myocutaneous flap was considered to be too bulky. The result was excellent. The gracilis muscle flap is very reliable and tends to be more reliable than the gracilis myocutaneous flap. It has been used successfully for other perineal defects.[12] Five of the six patients treated with gracilis myocutaneous flaps had complete healing. Others have had excellent success with gracilis myocutaneous flaps for radionecrosis of the vulva or distal vagina.[13-15] A total of six patients have been reported and all healed completely. The one patient in this series who did not heal had a huge defect and eventually required pelvic exenteration because of progressive necrosis. Two of the five patients with gracilis myocutaneous flaps that healed completely had some skin loss, which is common with gracilis myocutaneous flaps, and introital stenosis developed in both patients as a result of the skin loss.

Local full-thickness skin flaps have been used for radionecrosis of the vulva or perineum with reasonable success.[16, 17] In this series one patient had an excellent result with femoral fasciocutaneous flaps and local buttock flaps failed in one patient after failure of a gracilis myocutaneous flap. It is unlikely that these flaps are as reliable as the gracilis myocutaneous flaps. Nonetheless with good surgical judgment local flaps normally used for vulvar and perineal reconstruction after cancer surgery should be appropriate for coverage after radical excision of radionecrotic tissue.[18]

It is hoped that new local therapies will be developed that result in healing of these radionecrotic ulcers without surgery. Until then excision of all radiation-damaged tissue with appropriate tissue coverage will be the treatment of choice. It is possible that pelvic exenteration can be avoided by incomplete excision of the radiation-damaged tissue before coverage with myocutaneous flaps. The flap theoretically would accomplish biologic debridement. This has been advocated by Marino[19] with excellent results in terms of both healing and preservation of vital structures. We have no experience with this technique but it certainly deserves consideration. We did use hyperbaric oxygen in one patient without beneficial effect. It is possible that hyperbaric oxygen may help to heal smaller ulcers but we doubt its widespread use will significantly change the need for surgery for this problem.

Radionecrosis of the vulva or distal vagina is a difficult problem. Hesitation to subject the patient to extensive surgery is understandable. Unfortunately, delay in surgical treatment serves only to prolong the misery of the patient and may delay the diagnosis of recurrent cancer.

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Cocaine and the use of alcohol and other drugs during pregnancy

Ann P. Streissguth, PhD,[a] Therese M. Grant, MEd,[a] Helen M. Barr, MS,[a] Zane A. Brown, MD,[b] Joan C. Martin, PhD,[a] Dennis E. Mayock, MD,[c] Sharon Landesman Ramey, PhD,[d] and Leejon Moore, MD[a]

Seattle, Washington, and Birmingham, Alabama

Recent reports of adverse pregnancy outcomes associated with prenatal cocaine exposure have raised questions about the actual numbers of infants who are exposed to cocaine in utero. Whereas toxicologic urine screens obtained at delivery can detect cocaine use in the preceding few days, they fail to yield a comprehensive picture of use during and immediately before pregnancy. According to postpartum self-report, 15% of a teaching hospital sample and 3% of a private hospital sample of mothers had used cocaine during pregnancy or in the previous month (total = 876). Rates at the teaching hospital reflect a fifteenfold increase over the past 12-year period, when compared with previously obtained data. Cocaine users were significantly more likely to report that they drank alcohol, smoked cigarettes, and took other illicit drugs during pregnancy than women who denied using cocaine. Mothers at highest risk for cocaine use were those who were black (20%), were single-separated-divorced (24% to 33%), and had less than a high school education (21%). (AM J OBSTET GYNECOL 1991;164:1239-43.)

Key words: Cocaine prevalence in pregnancy, alcohol prevalence in pregnancy

Recent reports have indicated an alarming increase in the use of cocaine. Cocaine use has been described by the director of the National Institute on Drug Abuse as a "major public health threat."[1] Cocaine readily crosses the placenta[2]; therefore effects on the fetus are of grave concern. Several recent studies have described perinatal effects of cocaine abuse, including intrauterine growth retardation,[3-7] earlier gestational age at delivery,[3, 4] and increased abruptio placentae,[3, 8, 9] as well as cerebral infarction, abnormal electroencephalogram, and neurobehavioral signs in the neonatal period.[3, 5, 6, 9-11] Intrauterine growth retardation was not significantly associated with prenatal cocaine exposure in one study,[12] and the findings of abruptio placentae were not replicated in two studies.[4, 11] It is possible that some of these discrepancies relate to the use of other drugs among cocaine-using women or to unique sample characteristics of the mothers studied.

This study was undertaken for three reasons: (1) to compare the prevalence of self-reported cocaine and other drug use among mothers at several Seattle area hospitals, (2) to examine the relationship of cocaine use to demographic characteristics and to the use of other drugs, and (3) to compare cocaine use during pregnancy in 1987 with similar data from 1975.

Material and methods

Cocaine use during pregnancy and in the month or so before pregnancy was assessed by self-report on a one-page Hospital Screening Questionnaire (HSQ) developed for this study after extensive pretesting. Similar information was obtained for other illicit drugs, alcohol, and tobacco. The hospital screening questionnaire, which begins with a statement about the purpose of the study and the confidentiality of the responses, was distributed in the postpartum unit on the first morning after delivery at three hospitals: a teaching hospital that includes a high-risk inner city population, as well as low-risk, more highly educated mothers, and two private hospitals, one urban and one suburban, serving more middle-class populations. Data from the latter two hospitals were combined because they were similar. All consenting mothers were given questionnaires by trained research assistants who identified themselves as separate from the hospital staff and who informed mothers that their responses would not be entered into their medical records. At the teaching hos-

From the Department of Psychiatry and Behavioral Sciences,[a] the Department of Obstetrics and Gynecology,[b] and the Department of Pediatrics,[c] University of Washington School of Medicine, and the Civitan International Research Center, University of Alabama at Birmingham.[d]

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Reprint requests: Dr. Ann Streissguth, Pregnancy and Health Study (GG-20), University of Washington, 2707 NE Blakeley, Seattle, WA 98105.
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Table I. Self-reported drug use during pregnancy and in the month before pregnancy by mothers at teaching hospital versus private hospital ($N = 876$)

	Teaching hospital (n = 591)				*Private hospital (n = 285)*				*Test of differences*	
	P (%)	*D (%)*	*P + D (%)*	*95% Confidence interval for % P + D (%)*	*P (%)*	*D (%)*	*P + D (%)*	*95% Confidence interval for % P + D (%)*	*Teaching hospital vs private hospital*	*P vs D*
Any alcohol	51	43	57	52-62	58	46	61	55-67	—	†
Any binge alcohol	12	6	12	10-15	7	1	6	3-9	*	†
Any cigarettes	48	44	48	44-52	16	13	15	11-20	*	†
Any illegal drugs	29	24	32	28-36	9	6	10	6-13	*	†
Any marijuana	24	18	25	22-29	8	5	8	5-12	*	†
Any cocaine	13	11	15	12-18	2	1	3	<1-4	*	†
Other street drugs	8	6	10	8-12	1	<1	1	0-3	*	—
None of above (P or D)	29	38	25	21-30	39	49	35	29-40	*	†

P, Month or so prior to pregnancy; *D*, during pregnancy.

*Significance tests for difference in drug use between hospitals. Two-tailed tests for difference in proportions from two unpaired samples: $p \leq 0.05$.

†Significance tests for difference in drug use before pregnancy versus during pregnancy. Two-tailed tests for difference in correlated proportions: $p \leq 0.05$.

pital the compliance rate was 88% for a total of 591 questionnaires obtained. At the private hospitals the compliance rate was 80% for a total of 285 completed questionnaires. The 1974 and 1975 comparison data from the teaching hospital were available on 405 mothers who had participated in a previous study,[13] in which the participation rate was 85% and the data were obtained by self-report during midpregnancy.

Results

For the 1987 data, the reported tobacco and illicit drug use differed markedly between the two types of hospitals (Table I), and except for some street drugs, more women reported use in the month before pregnancy than during pregnancy. However, because there are uncertainties regarding the timing of conception and recall across this 9-month period, we combined data from these two time periods in preparing the remaining tables. At the teaching hospital 15% of the mothers reported cocaine use during pregnancy or the previous month; at the private hospitals the comparable figure was 3%. Overall alcohol use was more comparable across hospitals, but teaching hospital mothers reported twice as much binge drinking as mothers from the private hospitals. Reported use of alcohol and other drugs declined significantly from the "month prior" to during pregnancy, except for the category of "other street drugs," which includes drugs such as heroin, PCP, and amphetamine but not marijuana and cocaine.

Table II shows that, as anticipated, the demographic characteristics of the mothers at the two hospitals are very different. Table III, which combines mothers from both hospitals, shows that maternal drug use is related to demographic variables. White mothers more frequently reported drinking during pregnancy, whereas black mothers more frequently reported use of tobacco and cocaine. Cocaine use is lowest among married and more highly educated mothers. The highest risk groups for drug use and binge drinking during pregnancy are high school dropouts and unmarried mothers. Table III also reveals the percentage of babies who were not exposed to drugs, according to maternal report of alcohol, cigarette, and/or illicit drug use, either in the month before pregnancy or during pregnancy. Only 26% of white babies and 34% of black babies were reportedly drug and alcohol free. Maternal marital status was related to drug- and alcohol-free babies: 35% of babies of married mothers, 17% of those of single mothers, and 11% of those of divorced-separated mothers were reportedly drug and alcohol free. Mothers who reported cocaine use were also more likely to report use of alcohol and other drugs than were cocaine abstainers (Table IV). We can see from Table IV that it is virtually impossible to study the effects of cocaine in the complete absence of other drugs, at least in this sample.

This study demonstrates that the dramatic increase in cocaine use in the general population over the past few years holds true for pregnant women as well. There was a fifteenfold increase in reported cocaine use at the teaching hospital during the 12-year period described (Table V). This change cannot be accounted for by demographic changes in the hospital population, as the teaching hospital population had relatively comparable demographics for the two time periods, except for a higher proportion of older and single mothers, a trend

Table II. Demographic characteristics of mothers at teaching hospital versus private hospital

	Teaching hospital (n = 591)			*Private hospital (n = 285)*		
	No.	*%*	*Mean ± SD*	*No.*	*%*	*Mean ± SD*
Race*						
White	388	67		260	94	
Black	109	19		1	<1	
Other	81	14		17	6	
Marital status*						
Married	281	51		264	94	
Single	235	42		10	4	
Other	40	7		6	2	
Parity*						
Primiparous	279	50		99	36	
Multiparous	285	51		177	64	
Education* (yr)			12.24 ± 2.40			14.30 ± 2.13
Age* (yr)			24.69 ± 5.79			29.83 ± 4.44

*$p \leq 0.05$. Significance test for differences in race, marital status, and parity: Two-tailed χ^2 test for differences in proportions. Significance test for difference in years of education and maternal age in years: Two-tailed approximate t on basis of separate variance estimates.

Table III. Percent drug use by demographic categories: 1987 Sample from three hospitals (N = 876)

Demographic category	*No.*	*Alcohol*		*Cigarettes (%)*	*Illegal drugs*				*None of these (%)*
		Any (%)	*Binge (%)*		*Any (%)*	*Marijuana (%)*	*Cocaine (%)*	*Other (%)*	
Race									
White	648	65	11	36	24	19	9	8	26
Black	110	41	9	52	36	27	20	5	34
Other	98	45	9	35	19	15	11	6	40
Age (yr)									
14-19	130	40	16	57	32	29	14	12	36
20-29	475	63	12	42	28	22	13	6	25
30-42	262	60	5	18	15	11	5	7	34
Education (yr)									
6-11	210	53	15	68	40	33	21	12	20
12	259	50	9	41	27	19	12	9	32
13-15	220	67	12	29	22	16	9	6	29
16-20	177	67	5	8	8	7	<1	<1	33
Marital status									
Married	545	58	6	23	13	10	4	4	35
Single	245	61	19	63	46	38	24	14	17
Other	46	73	20	70	48	30	33	17	11
Parity									
Primiparous	378	59	14	37	26	22	10	7	29
Multiparous	462	58	8	37	24	18	12	7	30

Last column (labeled *None of these*) means no alcohol *and* no cigarettes *and* no marijuana *and* no cocaine *and* no other illegal "street" drugs.

that is ongoing at most obstetric centers.[14] Reported alcohol use decreased by 26% during pregnancy, whereas binge drinking (five or more drinks at a time) decreased to half of its 1975 level. The use of "other" illicit drugs (heroin, methadone, and amphetamines in about equal proportions) shows a fivefold increase over this 12-year period, while tobacco and marijuana use has remained relatively stable. Nevertheless, "any" alcohol (at 57%) and cigarettes (at 48%) were the two drugs most frequently reported as being used during pregnancy. We note that, although there were twice the percentage of women who reported no drug use in 1987 as compared with 1974, this still accounted for only 25% of the teaching hospital sample.

Comment

This study reveals that 15% of the women delivered of their infants at a teaching hospital reported using cocaine at some point during pregnancy or in the previous month. This represents a fifteenfold increase in cocaine use at this hospital over a 12-year period (see Fig. 1). Cocaine use was highest among women who were black, poorly educated, or single-divorced-separated. This is in contrast to recent data from the National Household

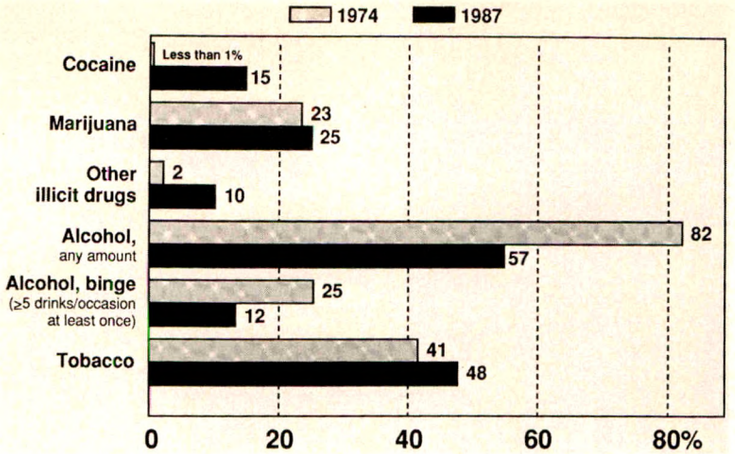


Fig. 1. This figure shows changes in self-reported use of cocaine, alcohol, and other drugs for 1975 versus 1987 at teaching hospital only. Data here combine two time periods during pregnancy and just before pregnancy or pregnancy recognition. *Other illicit drugs* includes heroin, methadone, amphetamines, and all other illicit drugs except cocaine and marijuana.

Table IV. Use of drugs by cocaine users versus abstainers (N = 876)

	Abstainers (n = 777)		*Cocaine users (n = 97)*		*Users vs abstainers (test of differences)*
	No.	*%*	*No.*	*%*	
Any alcohol	350	56	67	82	*
Any binge alcohol (≥5 drinks)	62	8	29	30	*
Any cigarettes	240	31	86	89	*
Any marijuana	106	14	65	68	*
Other street drugs (excludes marijuana, cocaine)	30	4	32	33	*

Two of the 876 patients provided no information on cocaine. The denominators in these columns are smaller than indicated by number of identified cocaine users or abstainers because some questions were not asked on the first editions of these pilot study questionnaires or some patients did not provide answers for all information requested.

*χ^2 tests of statistical significance, $p \leq 0.05$.

Table V. Drug use at teaching hospital in 1975 versus 1987

	1975 (n = 405)		*1987 (n = 591)*	
	%	*95% Confidence interval (%)*	*%*	*95% Confidence interval (%)*
Any alcohol	83	79-86	57	52-62
Any binge alcohol	25	21-29	12	10-15
Any cigarettes	41	36-46	48	44-52
Any illegal drugs	23	19-27	32	28-36
Any marijuana	23	19-27	25	22-29
Any cocaine	<1	0-1	15	12-18
Other street drugs	2	0-4	10	8-12
None of above	13	10-16	25	21-30

Drug Survey,[15] which found a 3.5% rate of cocaine use "during the past month" among black and white women of childbearing age who were not pregnant. The prevalence figures from the teaching hospital do not generalize well to the private hospitals, which may be due in part to demographic differences in the patient populations. Approximately 3% of the mothers at private hospitals reported cocaine use during pregnancy.

Alcohol use has decreased significantly over the past 12 years in our study; however, a recent report from Texas cites a twofold increase in alcohol abuse (defined as ≥2 ounces of absolute alcohol per day, on average) between 1977 to 1980 and 1987.[16] In our 1987 study the highest use was among white mothers, and the highest binge drinking rate was among white mothers and teenagers. In contrast to the demographics associated with cocaine use, binge drinking was reported more frequently among mothers without a high school education and those with some college (Table III). This pattern of reported use of five or more drinks per occasion has recently been associated with significantly lower IQ scores and more learning problems in exposed 7-year-old children.[17]

Mothers who reported using cocaine during pregnancy also were more likely to use alcohol and other drugs. This suggests that for studies of cocaine teratogenesis, assessment of other drug exposures must be carefully integrated into the study design. This is particularly true for marijuana, which was used by 68% of the cocaine users but only 14% of the cocaine abstainers, and binge alcohol, which is the drinking pattern for 30% of the cocaine users but only 8% of the cocaine abstainers.

The widespread increase in reported cocaine use

during pregnancy or in the previous month demonstrated in this study, as well as the frequent combination of cocaine with alcohol and other drugs of abuse, indicates that a large number of infants are born drug exposed. At the teaching hospital 75% of the babies were apparently exposed to alcohol, tobacco, and/or illicit drugs; 32% were exposed to illicit drugs alone (Table I).

The long-term consequences associated with prenatal alcohol use are well documented,[18] but little is known about the ultimate effects of prenatal cocaine exposure, although warnings about short-term effects have been issued.[19] An unpublished pilot study from our unit indicates some developmental delay and considerable social upheaval in the lives of babies exposed to cocaine in utero; only 45% of the babies born to cocaine-using mothers were still in the care of their biologic mothers at 6½ months of age. However, identification of cocaine as a correlate of infant outcomes independent of other covariates was not possible because of confounding with social factors, reported exposure to other drugs, and small sample size. It is essential that long-term follow-up studies be undertaken to examine the neurobehavioral consequences of prenatal cocaine exposure. Recent research from animal laboratories suggests that prenatal cocaine exposure does alter central nervous system development.[20, 21]

This study indicates that hospital screening of postpartum women is a useful method of potentially identifying infants exposed prenatally to alcohol, tobacco, and illegal drugs. Drug testing of all mothers during pregnancy may not be feasible and urine screening of all newborns can be fraught with medical-legal problems; therefore this questionnaire approach provides a reasonable alternative to identify high-risk mothers and babies at delivery. This self-report questionnaire could be adapted effectively for pregnant women as well. Knowledge about prenatal alcohol and drug exposure is an essential step in prevention and intervention efforts.

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Fetal thyroid-stimulating hormone response to maternal administration of thyrotropin-releasing hormone

J.G. Thorpe-Beeston, MD,[a] K.H. Nicolaides, MD,[a] R.J.M. Snijders, MSc,[b] J. Butler, MA,[b] and A.M. McGregor, MD[c]

London, England

Thyroid-stimulating hormone was measured in fetal blood samples obtained by cordocentesis before and after blood transfusion in Rh-affected pregnancies at 25 to 37 weeks' gestation. In eight of 26 study subjects thyrotropin-releasing hormone was given to the mothers immediately after the pretransfusion samples were obtained. In this group the fetal thyroid-stimulating hormone concentrations in the posttransfusion samples were significantly higher than those before the transfusion. (AM J OBSTET GYNECOL 1991;164:1244-5.)

Key words: Thyroid function, cordocentesis, respiratory distress syndrome

Animal studies have shown improvement in fetal pulmonary maturation after the maternal administration of thyrotropin-releasing hormone.[1] In human beings, intravenous injection of thyrotropin-releasing hormone to pregnant women within 3 hours before delivery at term was associated with increased thyroid-stimulating hormone concentrations in cord blood at delivery.[2, 3] The aim of this study was to investigate whether maternal thyrotropin-releasing hormone, administered at earlier gestations, could also stimulate the fetal pituitary.

Patients and methods

Serum thyroid-stimulating hormone was measured by radioimmunoassay in maternal and umbilical venous blood samples[4] obtained immediately before and after fetal blood transfusion by cordocentesis,[5] in 26 red blood cell isoimmunized pregnancies at 25 to 37 weeks' gestation. In eight patients, immediately after obtaining the pretransfusion fetal blood sample, thyrotropin-releasing hormone (200 μg) was given intravenously to the mothers.

Results

In the eight patients who were given thyrotropin-releasing hormone, gestation (mean = 32, SD = 2 weeks), fetal hemoglobin increase as a result of the transfusion (mean = 6.5, SD = 1.2 gm/dl), expansion of the fetoplacental blood volume[5] (mean = 70, SD = 24%), and rate of transfusion (mean = 8.7, SD = 2.9 ml/min) were not significantly different from the corresponding values in the 18 patients who were not given thyrotropin-releasing hormone (mean = 30, SD = 3 weeks, $t = -1.87$; mean = 6.6, SD = 2.4 gm/dl, $t = -0.03$; mean = 97, SD = 63%, $t = 1.12$; mean = 10.8, SD = 3.9 ml/min, $t = 2.28$, respectively).

In the group that received thyrotropin-releasing hormone, the mean thyroid-stimulating hormone concentration in the posttransfusion samples was significantly higher than that before the transfusion both in maternal (Fig.1; mean difference = 7.687 mU/L, $t = 6.04$, $p < 0.001$) and fetal blood (Fig. 1; mean difference = 41.929 mU/L, $t = 6.27$, $p < 0.01$). The percentage increase in fetal thyroid-stimulating hormone concentration was significantly related to the time interval between the administration of thyrotropin-releasing hormone and the collection of the posttransfusion samples (Fig. 1; $r = 0.832$, $n = 8$, $p < 0.05$), but not to gestation at transfusion ($r = 0.092$), the change in fetal hemoglobin concentration ($r = 0.03$), the rate of transfusion ($r = 0.228$), or expansion of the fetoplacental blood volume ($r = 0.427$). In the control group there were no significant differences between the pretransfusion and posttransfusion thyroid-stimulating hormone concentrations in either the fetal (mean difference = 1.317, $t = 1.03$) or maternal blood (mean differences = 0.13, $t = 1.11$).

Comment

Fetal blood transfusion does not change the fetal thyroid-stimulating hormone concentration. However, maternal administration of thyrotropin-releasing hormone is associated with a rapid increase in fetal thyroid-stimulating hormone. Because the magnitude of the

From the Harris Birthright Research Centre for Fetal Medicine[a] and the Departments of Clinical Biochemistry[b] and Medicine,[c] King's College School of Medicine and Dentistry.
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Reprint requests: K. H. Nicolaides, MD, King's College School of Medicine and Dentistry, Denmark Hill, London, United Kingdom SE5 8RX.
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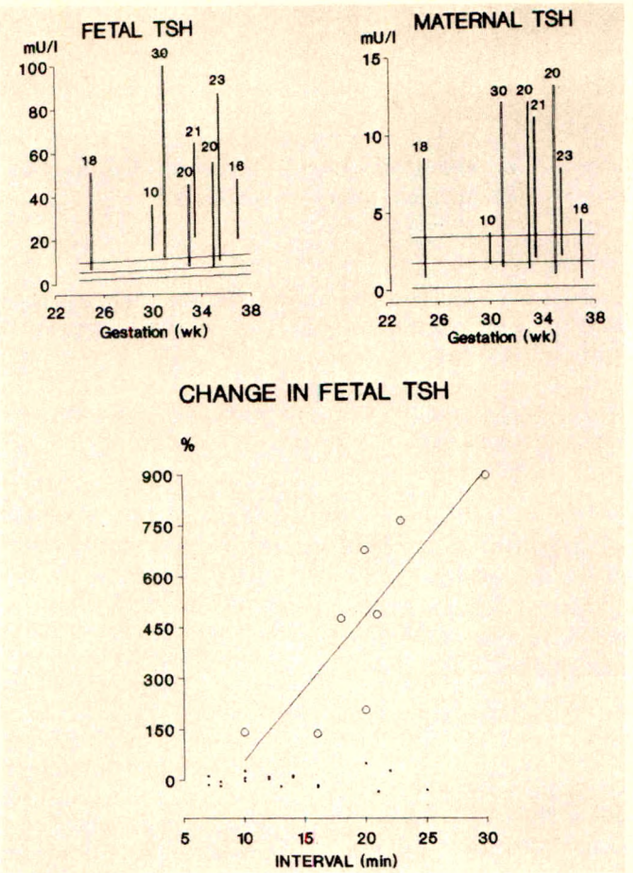


Fig. 1. Increase in fetal and maternal blood concentration of thyroid-stimulating hormone (mU/L) within 10 to 30 minutes from administration of thyrotropin-releasing hormone (actual time interval given above thyroid-stimulating hormone response in each case), plotted on appropriate reference range (mean, 5th, and 95th percentiles) for gestation *(top)*. Change in fetal thyroid-stimulating hormone is significantly related to time interval from administration of thyrotropin-releasing hormone to mother (o); in control fetuses (.) there was no significant difference between pretransfusion and posttransfusion thyroid-stimulating hormone concentrations.

fetal response is similar to that reported in fetuses undergoing thyrotropin-releasing hormone stimulation at term,[2, 3] the data of the present study suggest that fetal pituitary responsiveness is established from at least 25 weeks' gestation. Furthermore, the fetal response to thyrotropin-releasing hormone is much greater than the maternal response, and this increased sensitivity may be a consequence of reduced negative feedback on the pituitary because the fetal thyroid hormone concentration is lower than that in postnatal life.[4]

Further study is necessary to investigate the reproducibility of these findings in normal pregnancies and to define the possible effect of the increased fetal thyroid-stimulating hormone on the fetal thyroid gland, the time course of this response, and whether maternal thyrotropin-releasing hormone, unlike corticosteroids, can enhance fetal lung maturation from as early as 25 weeks' gestation.

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In utero diagnosis and management of fetal subdural hematoma

Siegfried Rotmensch, MD, Peter A. Grannum, MD, José A. Nores, MD, Cynthia Hall, MD, Marc S. Keller, MD, Shirley McCarthy, MD, and John C. Hobbins, MD

New Haven, Connecticut

The in utero ultrasonographic diagnosis of a fetal subdural hematoma is presented. The value of percutaneous umbilical blood sampling, transvaginal ultrasonography, and magnetic resonance imaging for diagnosis and management is discussed. (AM J OBSTET GYNECOL 1991;164:1246-8.)

Key words: Subdural hematoma, percutaneous umbilical blood sampling, transvaginal ultrasonography, magnetic resonance imaging

The in utero diagnosis of subdural hematoma has been reported in two cases.[1, 2] Percutaneous umbilical blood sampling, transvaginal ultrasonography, and magnetic resonance imaging have added new dimensions to the identification and management of this entity. We report a case and suggest guidelines for management.

Case history

A 37-year-old Haitian woman, gravida 4, para 3, was first seen at 32 weeks' gestation. Physical examination revealed a uterine fundal height of 37 cm. A transabdominal ultrasonographic scan showed a single live fetus in vertex presentation with moderate polyhydramnios. Biometry of the long bones was consistent with 32 weeks' gestation. Gross fetal anatomy appeared normal except for the intracranium, which could not be well visualized because of engagement of the fetal head in the pelvis. The biparietal diameter was consistent with a gestation of 37 weeks 5 days.

A transvaginal scan showed a grossly abnormal intracranial anatomy (Fig. 1). The brain was displaced from the inner table of the cranium by a hypoechoic extraaxial fluid collection. Within this extraaxial fluid, two hyperechoic irregular masses were seen in the right frontoparietal area. The right hemisphere and falx were displaced toward the left. The ventricular system was not dilated, and there was no evidence of intraventricular hemorrhage. The frontal portion of the lateral ventricles and the hippocampal gyri appeared to be symmetrically compressed by the extraaxial fluid. Interestingly, the sulci of the brain tissue were markedly accentuated and appeared as bright echogenic lines. The central areas of the brain tissue, which correlated with the anatomic location of white matter, appeared significantly more echogenic in comparison with the peripheral brain tissue, raising suspicion of evolving infarction or encephalomalacia. The cerebellar vermis and hemispheres could not be clearly outlined and appeared as an irregularly shaped mass in the posterior fossa.

The above findings were suggestive of a fetal subdural hematoma with multiple blood clots in the subdural space and secondary compression of brain tissue.

Percutaneous umbilical blood sampling was performed for chromosome analysis, fetal acid-base status, and coagulation profile. The following results were obtained: pH, 7.35; base deficit, −8.2 mmol/L; hematocrit, 14.1%, prothrombin time, 64.6 seconds (control, 11.1 seconds); partial thromboplastin time, >2 minutes (control, 28.6 seconds), platelet count, 236,000/ml; and a weakly positive direct Coomb's test. Fetal liver function tests and plasma protein level were within normal limits. The mother's blood type was O negative with an anti-D titer of 1:64. Screening tests for cytomegalovirus and toxoplasmosis were negative.

An intrauterine transfusion of 130 ml of O-negative packed red blood cells was performed, which raised fetal hematocrit to 30%. Because of the unexplained fetal coagulopathy, 30 ml of fresh frozen plasma was transfused empirically. After the transfusion the nonstress test became reactive for the first time, and the biophysical profile scored 10 points on a scale of 10. Amniocentesis revealed mature lung indices. The fetal karyotype was normal 46,XY.

To further characterize the intracranial findings, a magnetic resonance imaging scan of the fetal brain was obtained, which confirmed the diagnosis (Fig. 2). Fetal paralysis during the scan was achieved with pancuronium bromide, 0.1 mg/kg, via cordocentesis.

In consultation with the neonatologists, delivery was delayed despite mature fetal lung indices, to reduce the potential for other complications of prematurity. Nine days after the intrauterine transfusion, the daily nonstress test became nonreactive again, and the biophys-

From the Section of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, and the Department of Diagnostic Imaging, Yale University School of Medicine.

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Reprint requests: Siegfried Rotmensch, MD, Section of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510.

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ical profile failed to reveal breathing movements and tone. Marked polyhydramnios had acutely formed. An emergency low-transverse cesarean section was performed, and a male fetus weighing 2890 gm with Apgar scores of 3 and 5 at 1 and 5 minutes, respectively, was delivered. The umbilical cord arterial pH was 7.19 with a base deficit of −8.0 mmol/L. Marked craniomegaly with widespread sutures and bulging fontanelles were noted. The immediate neonatal course was complicated by multiple episodes of bradycardia. Respiratory efforts were essentially absent, leading to intubation. Neonatal brain scans by both ultrasonography and computed tomography revealed a large subdural hematoma, diffuse cerebral edema, and massive bilateral intraventricular and periventricular hemorrhages. The infant died 6 hours after delivery. Autopsy confirmed the diagnosis of bilateral subdural hematomas with multiple organized clots in the subdural space and massive intraventricular hemorrhage.

Comment

The antenatal diagnosis of a subdural hematoma can be based on the findings described. Transvaginal ultrasonography was of great value in complete visualization of the cranium in this fetus with vertex presentation, and magnetic resonance imaging was a useful adjunct for confirmation. Fetal paralysis with pancuronium via cordocentesis allowed for diagnostic magnetic resonance imaging without interference by fetal motion. We believe that the enhanced echogenicity of sulci and the apparent differential echogenicity of white and gray matter in our case reflect brain edema. Furthermore, the rim of fluid surrounding the brain in the subdural space might have provided an additional acoustic window, resulting in accentuation of the brain texture.

The prognosis for a fetus affected by subdural hematoma is conceivably dependent on the severity of the hemorrhage and the underlying cause. In one of the previously reported cases hypotonic quadriparesis and bilateral optic atrophy subsequently developed in the surviving infant.[2] In another case the fetus survived but experienced neonatal seizures.[1] The prognosis thus far appears to be dismal.

Our experience with fetal hydrocephalus has taught us that brain function cannot be predicted accurately by ultrasonographic findings except in the most severe cases. In the absence of extensive experience with fetal subdural hematoma, it appears prudent to optimize antenatal treatment and delivery management in the expectation of fetal survival. In contrast to the management of our own case, we suggest delivery as soon as fetal lung maturity is determined. In this case fetal intraventricular hemorrhage must have occurred in the 24-hour period before delivery or immediately post partum, since daily ultrasonographic scans showed no evidence of this complication. Earlier delivery might have prevented the occurrence of intraventricular hemorrhage.

If indicators of lung maturity are negative, we suggest percutaneous umbilical blood sampling for diagnosis and possible intrauterine transfusion. Percutaneous umbilical blood sampling revealed a severe co-

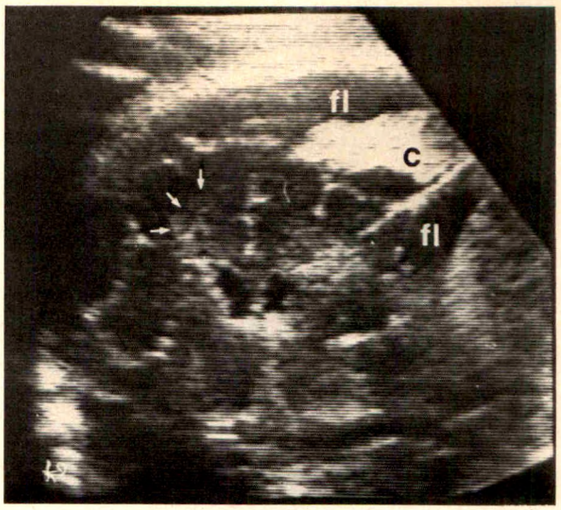


Fig. 1. Transvaginal coronal scan of fetal cranium. Large clot *(c)* and fluid *(fl)* in subdural space are present. *Arrows* point toward area of central echodensity, which is consistent with anatomic location of white matter surrounded by more echolucent gray matter.

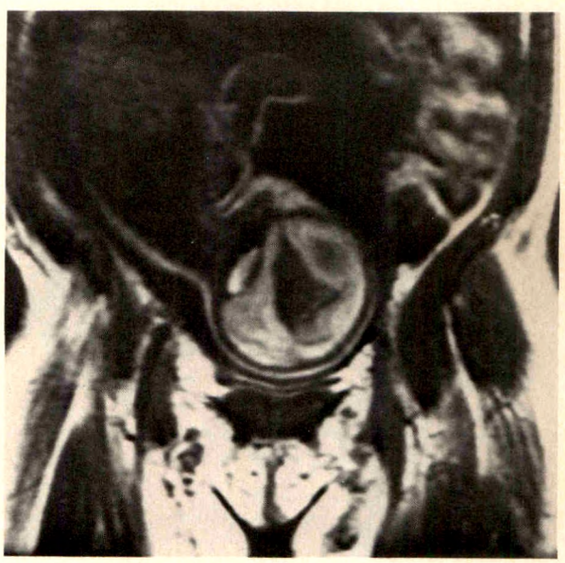


Fig. 2. T1-weighted (repetition time 500 msec; echo time 20 msec) magnetic resonance imaging in the coronal plane of mother but in sagittal plane of fetal head. In this sequence brightest signals are obtained from blood and fat. Fetal subdural hematoma surrounds the brain, whereas subcutaneous fat outlines external surface of fetus.

agulopathy and anemia in this case. Both were partially corrected by transfusion of donor blood cells and fresh-frozen plasma. Even though postransfusion clotting function could not be obtained, the coagulation function and hematocrit were improved 9 days later, at the time of delivery. Fetal blood specimens were not sufficient for a complete hematologic evaluation.

The route of delivery was by cesarean section in all three reported cases, because of remarkable enlargement of the head size. Undoubtedly, the overall efforts and maternal risks might appear excessive, considering the dismal outcome in at least two of the three cases which have so far been reported. However, physicians are ethically committed to optimize treatment in cases of a central nervous system lesion with yet undetermined outcome.[3]

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Calcium-regulating hormones and osteocalcin levels during pregnancy: A longitudinal study

Katsuyoshi Seki, MD, Noriko Makimura, DMSc, Chieko Mitsui, BS, Junko Hirata, BS, and Ichiro Nagata, MD

Tokorozawa City, Saitama, Japan

We measured serum concentrations of calcium-regulating hormones and osteocalcin in 20 women longitudinally throughout pregnancy. 1,25-Dihydroxyvitamin D levels were high early in pregnancy and increased with advancing gestation. Parathyroid hormone and osteocalcin levels were low in early pregnancy. They declined toward the middle of pregnancy, but increased in late pregnancy. The serum osteocalcin level correlated with the parathyroid hormone level. The synthesis of osteocalcin by osteoblasts is stimulated by the action of 1,25-dihydroxyvitamin D, and serum osteocalcin levels are also related to the levels of parathyroid hormone. During early and mid pregnancy, the stimulatory effect of 1,25-dihydroxyvitamin D on the synthesis of osteocalcin may be overridden by the inhibitory effect of declining parathyroid hormone levels. The increase in osteocalcin level in late pregnancy may be a consequence of increasing levels of both parathyroid hormone and 1,25-dihydroxyvitamin D. (Am J Obstet Gynecol 1991;164:1248-52.)

Key words: Calcium-regulating hormone, osteocalcin, pregnancy

The effect of pregnancy on the maternal calcium content is unclear.[1] The issue has been investigated by means of photon absorptiometry or x-ray spectrophotometry, but the results appear contradictory. Osteocalcin is a bone-specific protein released into the circulation proportional to the rate of new bone formation.[2] The usefulness of serum osteocalcin measurements as a clinical marker of bone turnover is becoming more widely accepted.[3, 4] Serum osteocalcin levels are lower in pregnant women compared with nonpregnant women, indicating reduced bone turnover during pregnancy.[5] However, the cause of the decrease in osteocalcin levels during pregnancy is unclear. The synthesis of osteocalcin by osteoblasts is stimulated by the action of 1,25-dihydroxyvitamin D [$1,25(OH)_2D$],[6] and serum osteocalcin levels also are influenced by parathyroid hormone.[3] Serum $1,25(OH)_2D$ is increased early in pregnancy and continues to rise until delivery.[7]

From the Department of Obstetrics and Gynecology, National Defense Medical College.
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Reprint requests: Katsuyoshi Seki, MD, Department of Obstetrics and Gynecology, National Defense Medical College, Namiki 3-2, Tokorozawa, Saitama 359, Japan.
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The relationship between serum osteocalcin and parathyroid hormone has been studied by two groups of investigators. In women during midpregnancy, serum osteocalcin levels were low in both reports, whereas serum parathyroid hormone levels were high in one[5] and were not significantly different in the other, when compared with nonpregnant women.[8] These findings suggest that factors other than parathyroid hormone or $1,25(OH)_2D$ are likely to be responsible for the decreased osteocalcin level during pregnancy.

However, there are also reports that indicate that parathyroid hormone levels in pregnant women may be lower than those in nonpregnant women and that serum intact parathyroid hormone may decline during pregnancy.[9, 10] If this is the case, low serum osteocalcin levels during pregnancy may be related to the decreased parathyroid hormone secretion. Serum osteocalcin and intact parathyroid hormone levels have not heretofore been measured in the same pregnant women. To further evaluate the changes in serum osteocalcin during pregnancy and to identify possible causes of the changes in osteocalcin levels, we measured blood levels of calcium, phosphorus, calcium-regulating hormones, and osteocalcin longitudinally throughout pregnancy.

Material and methods

The study population consisted of 20 women, ranging in age from 22 to 34 years. Twelve were nulliparous and eight were multiparous. All patients experienced uncomplicated pregnancies of duration between 38 and 41 weeks from the onset of the last menstruation. They received no medication except ferrous sulfate. Nineteen were delivered vaginally after normal labor, and one was delivered by cesarean section because of dysfunctional labor. All infants were normal at birth and weighed between 2.79 and 3.56 kg. All women breast-fed their infants through at least the first 6 weeks post partum.

Blood samples were collected by antecubital venipuncture in the morning, after an overnight fast. The initial samples were obtained on entry into the study before completion of 7 weeks from the onset of the last menstruation, and subsequent samples were collected at 4-week intervals throughout the remainder of gestation, on the third postpartum day, and during the sixth postpartum week.

Serum calcium levels were determined by atomic absorption spectrophotometry (model 603, Perkin-Elmer, Norwalk, Conn.) and phosphorus levels, by the Fiske and SubbaRow colorimetric procedure. Ionic calcium (Ca^{++}) activity was measured in a serum aliquot handled anaerobically with a NOVA 8 calcium analyzer (NOVA Biomedical, Waltham, Mass.).

Serum intact parathyroid hormone was determined with the commercially available immunoradiometric assay (Allegro-Nichols Institute, San Juan Capistrano, Calif.). This system is a two-site immunoradiometric assay, with two different, affinity-purified goat polyclonal antisera to human parathyroid hormone, one binding with the midregion and C-terminal portion of parathyroid hormone and a second binding with the N-terminal region; the latter is radiolabeled with iodine 125. The sensitivity of the assay was 1 pg/ml, and it is believed to accurately quantify the intact form of parathyroid hormone, PTH-(1-84).[11]

Serum calcitonin was measured by radioimmunoassay with kits obtained from Daiichi Radioisotope Laboratory (Tokyo), and serum osteocalcin was quantitated by radioimmunoassay with kits obtained from the Commissariat a l'Energie Atomique (Gif-sur-Yvette, France). The intraassay and interassay coefficients of variation were $<10\%$ in the assays for intact parathyroid hormone, calcitonin, and osteocalcin.

$1,25(OH)_2D$ was measured by radioreceptor assay after extraction and purification of the samples by column chromatography. The radioreceptor assay was performed as described by Eisman et al.[12] The sensitivity of the assay was 9 pg/ml, and the intraassay and interassay coefficients of variation were $<5\%$, respectively.

Statistical analyses were performed with analysis of variance, and regression analysis was done with repeated measures.

Results

Mean ($\pm$SD) levels of calcium, Ca^{++}, phosphorus, parathyroid hormone, calcitonin, $1,25(OH)_2D$, and osteocalcin during pregnancy and the puerperium are shown in Fig. 1. Calcium levels for weeks 20 to 23 through week ≥ 36 of pregnancy were significantly lower than those 6 weeks post partum, whereas those of week ≤ 7 through weeks 16 to 19 of pregnancy were not. The first-degree regression of mean calcium levels on duration of pregnancy was significant ($F = 65.6$, $p < 0.005$) (Fig. 1). Calcium levels 3 days post partum were significantly lower than those 6 weeks post partum.

Ca^{++} levels were not significantly different from those 6 weeks post partum throughout pregnancy (Fig. 1). Ca^{++} exhibited relatively little fluctuation during pregnancy, and neither first-degree ($F = 4.5$, not significant) nor second-degree ($F = 0.27$, not significant) regression provided a satisfactory fit. Ca^{++} levels were not significantly different between 3 days and 6 weeks post partum.

For weeks 28 to 31 through week ≥ 36 but not week ≤ 7 through weeks 24 to 27, the p levels were significantly lower than those 6 weeks post partum (Fig. 1). The p levels declined progressively throughout preg-

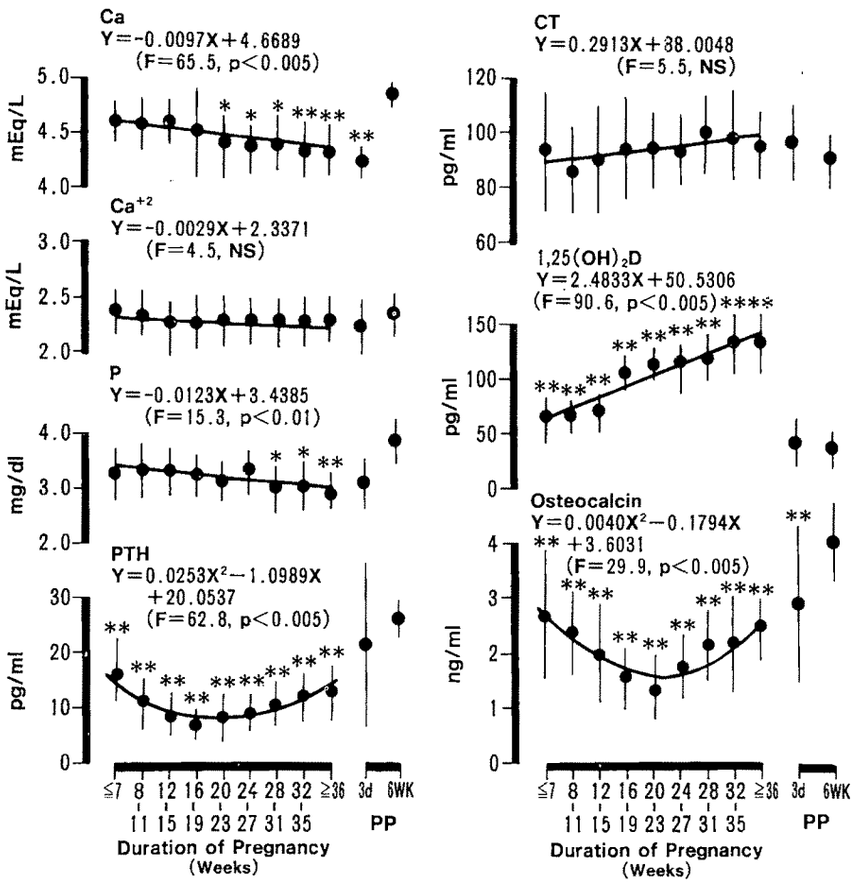


Fig. 1. Mean (±SD) levels of calcium *(Ca)*, Ca^{++} *(Ca^{+2})*, phosphorus *(P)*, parathyroid hormone *(PTH)*, calcitonin *(CT)*, $1,25(OH)_2D$, and osteocalcin during pregnancy and the puerperium *(PP)* and their regressions on duration of pregnancy (first degree: calcium, Ca^{++}, phosphorus, calcitonin, and $1,25(OH)_2D$; second degree: parathyroid hormone and osteocalcin). *Asterisk*, $p < 0.05$; *double asterisk*, $p < 0.01$ compared with 6 weeks post partum.

nancy, and the first-degree regression was significant ($F = 15.3$, $p < 0.01$). The p levels for 3 days post partum were not significantly different from those for 6 weeks post partum.

Parathyroid hormone levels were significantly lower than those 6 weeks post partum throughout pregnancy (Fig. 1). Levels declined toward the middle of pregnancy and increased thereafter. Although the first-degree regression was not significant ($F = 0.01$, not significant), a second-degree regression provided a satisfactory fit ($F = 62.8$, $p < 0.005$). Parathyroid hormone levels were not significantly different between 3 days and 6 weeks post partum.

Calcitonin levels were not significantly different compared with those 6 weeks post partum throughout pregnancy, and the first-degree regression was not significant ($F = 5.5$) (Fig. 1). Calcitonin levels on the third postpartum day were not significantly different from those 6 weeks post partum.

$1,25(OH)_2D$ levels were significantly higher than those 6 weeks post partum throughout pregnancy (Fig. 1). $1,25(OH)_2D$ levels increased during pregnancy, and the first-degree regression was highly significant ($F = 90.6$, $p < 0.005$). $1,25(OH)_2D$ levels for 3 weeks post partum were not significantly different from those for 6 weeks post partum.

Serum osteocalcin levels were significantly lower than those 6 weeks post partum throughout pregnancy (Fig. 1). Serum osteocalcin levels declined toward the middle of pregnancy and increased subsequently. Although the first-degree regression was not significant ($F = 0.15$), the second-degree regression was significant ($F = 29.9$, $p < 0.005$). Serum osteocalcin levels 3 days post partum were significantly lower than those 6 weeks post partum.

Osteocalcin levels correlated significantly with parathyroid hormone levels in 17 of the 20 women (correlation coefficients: 0.82, 0.88, 0.73, 0.68, 0.67, 0.91,

0.87, 0.86, 0.79, 0.75, 0.69, 0.77, 0.67, 0.67, 0.86, 0.90, 0.89) but not in the other 3 women (correlation coefficients: 0.57, 0.64, 0.61). Furthermore, mean osteocalcin levels correlated with mean parathyroid hormone levels during pregnancy (correlation coefficient, 0.91; $p < 0.005$).

Comment

Calcium and phosphorus in maternal serum declined during pregnancy. This is in keeping with the results of other reported studies.[1] Serum levels of Ca^{++} showed no significant change during pregnancy. Previous studies on Ca^{++} levels during pregnancy yielded contradictory results, with increasing and decreasing trends noted in different reports, both cross-sectional investigations with flow-through electrode analysis.[1] According to the longitudinal investigation of Pitkin et al.,[13] Ca^{++} values declined only slightly during pregnancy. Therefore the effect of pregnancy on serum Ca^{++} levels, if any, is considered minimal.

Most studies of parathyroid hormone, with traditional radioimmunoassays, have yielded results that support the concept of a physiologic hyperparathyroidism.[1] However, other investigators were unable to document a rise in serum parathyroid hormone in pregnancy, and a few reported that the levels may, in fact, be lower than those in nonpregnant women.[1, 10] These discrepancies arise, at least in part, from the limitations of traditional radioimmunoassays. Because of the presence of multiple species of predominantly inactive parathyroid hormone fragments, there is a marked discrepancy between total circulating levels of parathyroid hormone immunoactivity and bioactivity. Recently, Davis et al.[10] assessed the effect of pregnancy on parathyroid function using immunoradiometric assay for intact parathyroid hormone. They found a lower serum intact parathyroid hormone concentration in pregnant women compared with nonpregnant women, with lower values apparently as early as the first trimester. Although the results of our study are in general agreement with those of Davis et al., there are some discrepancies. They reported that the mean parathyroid hormone levels in the different trimesters were similar. In this study the levels declined toward the middle of pregnancy and increased thereafter. These discrepancies may be ascribed to the different investigational methods (cross-sectional vs longitudinal studies).

Previous studies on calcitonin secretion during pregnancy yielded inconsistent results.[1] We found no discernible change in calcitonin levels during pregnancy. This is in keeping with the data from a longitudinal study of Pitkin et al.,[13] in which no significant change in calcitonin levels was found. Thus the effect of pregnancy on calcitonin secretion, if any, may be minimal.

In agreement with the results of other reported studies,[1, 14, 15] $1,25(OH)_2D$ levels were high early in pregnancy and increased with advancing gestation. The principal physiologic factor promoting renal hydroxylation of 25(OH)D is parathyroid hormone. However, parathyroid hormone is not likely to be the cause of increased $1,25(OH)_2D$ formation, since parathyroid hormone levels were low during pregnancy. Therefore other factors may be responsible for the increased $1,25(OH)_2D$ level. In animals, some maternal serum $1,25(OH)_2D$ may be derived from the placenta. A capacity for 1α-hydroxylation of 25(OH)D has been demonstrated in the nephrectomized pregnant rat in vivo and in rat placenta and human decidua in vitro.[16-18] Breslau and Zerwekh[19] have demonstrated patients with pseudohypoparathyroidism who, while previously requiring vitamin D therapy to compensate for the impaired renal capacity to synthesize $1,25(OH)_2D$ were able to maintain normal serum $1,25(OH)_2D$ and calcium levels during pregnancy with no such treatment. The pregnancy-induced increase in circulating $1,25(OH)_2D$ may be the primary mediator of changes in maternal calcium metabolism.[9] Thus the increased $1,25(OH)_2D$ level may be the cause of decreased parathyroid hormone secretion during pregnancy.

It has been argued that the rise in $1,25(OH)_2D$ levels seen in early pregnancy is largely an artifact of an increase in the circulating concentration of the vitamin D-binding protein and that the free $1,25(OH)_2D$ index is not increased until late pregnancy.[15] However, Gertner et al.[9] demonstrated that pregnant women have physiologic absorptive hypercalciuria similar to that in patients with nephrolithiasis who have elevated serum $1,25(OH)_2D$ levels or in normal subjects given exogenous $1,25(OH)_2D$. It is not entirely clear whether pregnancy is accompanied by an increase in both bound and free $1,25(OH)_2D$.

Serum osteocalcin levels were low during pregnancy and lowest during midpregnancy. The findings are in general agreement with the results of other reported studies,[5, 8] and are compatible with a sequence in which bone turnover is reduced during early pregnancy, further decreases toward the middle of pregnancy, and increases in late pregnancy to the level of early pregnancy. The serum osteocalcin correlated with the parathyroid hormone level. This is in contrast to the results of Cole et al.,[5] who found no significant correlation between osteocalcin and parathyroid hormone. The discrepancy may be ascribed to the different immunoassays used for parathyroid hormone (traditional radioimmunoassay versus immunoradiometric assay for intact parathyroid hormone). Thus the low osteocalcin level during pregnancy may be related to the decreased parathyroid hormone level.

Parathyroid hormone and $1,25(OH)_2D$ can influence

the synthesis of osteocalcin. Parathyroid hormone produces an increase in bone resorption that, although it does not directly stimulate the synthesis of osteocalcin, results in an increase of the osteocalcin levels instead of increased parathyroid activity.[3] $1,25(OH)_2D$ stimulates the synthesis of osteocalcin by osteoblasts and promotes its release into the circulation.[4] Although $1,25(OH)_2D$ levels were high early in pregnancy and increased with advancing gestation, osteocalcin levels decreased toward the middle of pregnancy. Therefore the stimulatory effect of increasing $1,25(OH)_2D$ levels on the synthesis of osteocalcin may be overridden by the inhibitory effect of decreasing parathyroid hormone levels in early and midpregnancy. Serum osteocalcin levels increased in late pregnancy. This may be ascribed to the increase in both parathyroid hormone and $1,25(OH)_2D$ during this period. Free $1,25(OH)_2D$ has been reported to increase in late pregnancy.

Osteocalcin levels 6 weeks post partum were higher than those 3 days post partum, indicating an increased bone turnover in the late puerperium. Parathyroid hormone, calcitonin, and $1,25(OH)_2D$ levels were similar 3 days and 6 weeks post partum. However, parathyroid hormone levels were increased in both early and late puerperium. Thus it may be that long-term but not short-term parathyroid hormone stimulation leads to an increased production of osteocalcin by osteoblasts.

The changes in parathyroid hormone and osteocalcin levels during the puerperium may, in part, be ascribed to lactation. However, all women breast-fed their infants for at least 6 weeks post partum. Therefore we could not assess the effect of lactation per se on calcium-regulating hormones and osteocalcin levels during the puerperium.

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The relationship between autoantibodies and intrauterine growth retardation in hypertensive disorders of pregnancy

Albert El-Roeiy, MD, Stephen A. Myers, DO, and Norbert Gleicher, MD
Chicago, Illinois

Abnormal levels of autoantibodies have recently been demonstrated in patients with hypertensive disorders of pregnancy and patients whose fetuses have intrauterine growth retardation. We determined total immunoglobulin levels (immunoglobulin G, immunoglobulin M, and immunoglobulin A) and a broad panel of autoantibodies (six antiphospholipid, four antihistone, and four antipolynucleotide antibodies) in 50 normotensive pregnant females, 19 patients with preeclampsia, and 18 patients with chronic hypertension to examine the relationship to intrauterine growth retardation. Mothers who were delivered of infants with intrauterine growth retardation demonstrated significantly more autoantibody abnormalities in the two hypertensive groups and in the normotensive control group as compared with patients delivered of appropriately grown infants. The most frequently observed autoantibody abnormalities were antiphospholipid antibodies and the most frequently observed among those were immunoglobulin G isotypes. Total immunoglobulin levels in both hypertensive and normotensive groups were identical. These results suggest a close association between the degree of B-cell activation and both severity of hypertensive diseases and development of intrauterine growth retardation in their offspring. (Am J Obstet Gynecol 1991;164:1253-61.)

Key words: Autoantibodies, preeclampsia, intrauterine growth retardation

The possible involvement of the immune system in the pathophysiology of at least some hypertensive disorders of pregnancy has recently attracted increasing attention.[1] A number of investigators have suggested that some cases of preeclampsia-eclampsia are associated with clinical autoimmune phenomena.[2-5] Alterations in humoral immune function in such patients include production of antibodies toward trophoblast antigens,[6] amniotic glycoprotein, placental and renal antigens,[7] lymphocytes,[8] and laminin.[2]

A correlation between intrauterine growth retardation (IUGR) and the severity of a particular hypertensive disorder is well documented in the literature.[9, 10] A significant incidence of IUGR has also been observed in patients with such connective tissues diseases as systemic lupus erythematosus.[11] Lockshin et al.[12] suggested that IUGR in these patients may be related to disease activity, which in turn correlates to the presence of autoantibody abnormalities. These observations led us to consider whether alterations in fetal growth in patients with hypertensive diseases of pregnancy may be related to abnormal maternal autoantibody production. The aim of this study was to examine selective parameters of B-lymphocyte activity in patients with hypertensive disorders of pregnancy and their relationship to IUGR in their offspring.

From the Division of Reproductive Immunology and the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Mount Sinai Hospital Medical Center and Rush Medical College.
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Reprint requests: Albert El-Roeiy, MD, Department of Reproductive Medicine, University of California, San Diego, School of Medicine, La Jolla, CA 92093.
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Material and methods

Study population. Serum samples from 50 consecutive normotensive and apparently normal pregnant patients (control group) from 29 consecutive patients with preeclampsia (18 mild and 11 severe) and from 18 patients with chronic hypertension (three with superimposed preeclampsia) were studied. All but two patients with preeclampsia were primigravidas. Mild and severe preeclampsia, respectively, were defined according to the criteria of the American College of Obstetricians and Gynecologists.[13] The presence of any one or more of the following signs or symptoms marked the preeclampsia as severe: blood pressure of 160 mm Hg systolic and 110 mm Hg diastolic on two occasions at least 6 hours apart; proteinuria of at least 5 gm/24 hours; oliguria (urinary volume of <400 ml/24 hours); cyanosis or pulmonary edema. The diagnosis of chronic hypertension was based on a history of hypertension either before pregnancy or before 20 weeks' gestation. Fourteen of 18 patients (78%) with this diagnosis were receiving methyldopa therapy (Aldomet, 1.5 to 3 gm/day) at the time of the first prenatal visit.

Superimposed preeclampsia was diagnosed after the occurrence of either an abrupt rise of blood pressure (>30 mm Hg in systolic or 15 mm Hg in diastolic pres-

Table I. Demographic and clinical data in normotensive and hypertensive groups

	Maternal age (yr, mean ± SD)	Gravidity		Gestational age (wk, mean ± SD)	Race						Cigarette smokers	
					Black		White		Hispanic			
		Median	Range		n	%	n	%	n	%	n	%
Normotensive pregnant controls (n = 50)												
AGA (n = 43)	24.8 ± 11.2	2	1-5	39.9 ± 1.8	38	88	2	5	3	7	7	16
IUGR (n = 7)	24.2 ± 10.8	2	1-4	38.4 ± 2.1	6	86	1	14	—	—	2	28
Preeclampsia (n = 29)												
Mild (n = 18)												
AGA (n = 18)	24.6 ± 7	1	1-2*	36.6 ± 1.6	13	72	3	17	2	11	3	17
IUGR (n = 0)	—	—	—	—	—	—	—	—	—	—	—	—
Severe (n = 11)												
AGA (n = 6)	27 ± 12	1	1-2*	36.5 ± 3.4	3	50	2	33	1	17	1	16
IUGR (n = 5)	20.4 ± 6	1	1*	35.5 ± 3.5	3	60	1	20	1	20	1	20
Chronic hypertension (n = 18)												
AGA (n = 15)	30 ± 6*†‡	3	1-6†‡	36 ± 4.4	15	83	2	11	1	6	2	13
IUGR (n = 3)	25 ± 5	3	1-5†‡	37.6 ± 4	3	100	—	—	—	—	0	0

BP, Blood pressure.
*Significantly different from normotensive group ($p < 0.05$).
†Significantly different from mild preeclampsia group ($p < 0.05$).
‡Significantly different from severe preeclampsia group ($p < 0.05$).

sure) measured on two occasions 6 hours apart, or after a significant increase in the degree of proteinuria (>5 gm/24 hours), or both. Blood samples from all normotensive and hypertensive patients were drawn in labor within hours of delivery.

Normotensive control patients included 43 prospectively enrolled women delivered of infants with weights appropriate for gestational age (AGA) and seven patients delivered of infants with idiopathic IUGR. Control patients experienced no significant antepartum or intrapartum pregnancy complications. Neither study nor control patients showed any evidence of diabetes mellitus, rhesus isoimmunization, history or symptoms of autoimmune disease, chronic inflammatory disease, or drug abuse.

IUGR was defined as a gestational age–controlled birth weight below the 10th percentile of 2200 deliveries at Mount Sinai Hospital Medical Center in Chicago. Careful attention was paid to the accurate determination of correct gestational ages by reliable establishment of dates of last normal menses or ultrasonographic dating. Proteinuria was quantified by 24-hour urine collection in 14 of 29 patients with preeclampsia and four of 18 patients with chronic hypertension. The remaining patients underwent a semiquantitative assessment of proteinuria with a standard dipstick method (Ames Division, Miles Laboratories, Inc., Elkhart, Ind.). Blood urea nitrogen, creatinine, and uric acid were determined with a standard hospital autoanalyzer. Total platelet concentrations were established with a Coulter counter. Thrombocytopenia was defined as <100,000 platelets/ml and was verified by repeated sampling. All blood pressure levels were determined with a sphygmomanometer by recording the first and the fourth sounds as systolic and diastolic pressures. Mean arterial blood pressure levels were calculated from the systolic and diastolic blood pressures (diastolic + (systolic − diastolic)/3).

Immunologic studies. Total immunoglobulin G (IgG), total immunoglobulin M (IgM), and total immunoglobulin A (IgA) concentrations were measured with a routine radial immunodiffusion method.[14] The

Drug abuse					Proteinuria (mg/dl)				Creatinine (mg/dl)	
n	%	Systolic BP (mm Hg, mean ± SD)	Diastolic BP (mm Hg, mean ± SD)	Mean BP (mm Hg, mean ± SD)	Median	Range	Platelets (10^3/ml)	Uric acid (mg/dl, mean ± SD)	Median	Range
0	0	107 ± 12	61 ± 7	76 ± 10	—	—	265 ± 82	—	0.6	0.4-0.9
0	0	103 ± 10	68 ± 8	72 ± 11	—	—	245 ± 94	—	0.7	0.4-1
1	6	155 ± 9*	92 ± 12*	144 ± 15*	0.3	0.05	203 ± 86	6.6 ± 1	0.7	0.5-1.
—	—	—	—	—	—	—	—	—	—	—
0	0	163 ± 11*	101 ± 11*	121 ± 11*	0.4	0.3-0.5	160 ± 65*†	7.1 ± 1.2	0.9	0.5-1.3
0	0	152 ± 11*	92 ± 4*	112 ± 6*	1.71	1-3†	163 ± 63*†	6.4 ± 1	0.9	0.4-1.4
0	0	154 ± 18*	90 ± 11*	112 ± 13*	0.4	0.1-0.4	228 ± 73	5 ± 0.7	0.8	0.5-1.2
0	0	156 ± 20*	101 ± 8*	120 ± 12*	1.7	1.2-11†	172 ± 105	5.8 ± 0.4	0.9	0.4-1.3

autoantibody profile included autoantibodies to six phospholipids (cardiolipin, phosphatidylserine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid), total histone (H1), and three histone subfractions (H2A, H3, and H4) and to four polynucleotides (single-stranded deoxyribonucleic acid [DNA], double-stranded DNA, polyinosinic acid, and polythymidylic acid). All autoantibodies were separately evaluated for IgG, IgM, and IgA isotypes. After venipuncture, all samples were allowed to clot, were centrifuged at 2500 *g* for 15 minutes, and then the serum samples were decanted. All serum samples were stored below −70° C and assayed simultaneously at the completion of the study. A detailed description of autoantibody assays has been previously reported.[15]

Briefly, microtiter plates were coated with the different antigens and blocked for nonspecific binding. Duplicate serum samples were diluted to 1:150 in phosphate-buffered saline solution with 10% fetal calf serum and were added and incubated for 12 hours at 4° C. Affinity-purified, alkaline phosphatase–conjugated goat antihuman IgG, IgM, and IgA (Sigma Chemical Co., St. Louis) in phosphate-buffered saline solution with 10% fetal calf serum were added. The plates were then washed and 1 mg/ml of *p*-nitrophenyl disodium phosphate (Sigma) in 1 mol/L diethanolamine and 0.5 mol/L magnesium chloride at pH 9.6 was added as substrate. The plates were fixed and read at 405 nm with a spectrophotometer (Biotek Inc., San Francisco). Control serum samples with elevated IgG and IgM antiphospholipid, histone, and polynucleotide antibodies as well as a blank well with no serum were assayed in parallel with each plate. An abnormal result was defined as an optical density above the 99% confidence interval for each autoantibody and isotype. This interval was determined at different gestational ages from 43 normotensive prospectively followed up control patients delivered of AGA infants. Seven normotensive patients delivered of infants with IUGR were excluded from establishment of the confidence interval.

Statistical analysis. We compared age, gravidity, gestational age, systolic/diastolic pressures, mean blood pressure, proteinuria, uric acid, creatinine values, and platelet counts between control and study groups with the Tukey-Kramer test.[16] The test corrects for an unequal number of patients in each group and for multiple comparisons.

With the assumption of complete independence be-

Table II. Total immunoglobulin levels in normotensive and hypertensive pregnancies with and without IUGR

	IgG (mg/dl)	*IgM (mg/dl)*	*IgA (mg/dl)*
Normotensive pregnant controls (n = 50)			
AGA (n = 43)	939 ± 280	151 ± 55	159 ± 59
IUGR (n = 7)	910 ± 301	140 ± 69	135 ± 67
Preeclampsia (n = 29)			
Mild (n = 18)			
AGA (n = 18)	922 ± 244	134 ± 29	133 ± 28
IUGR (n = 0)			
Severe (n = 11)			
AGA (n = 6)	861 ± 220	129 ± 36	141 ± 34
IUGR (n = 5)	880 ± 241	159 ± 43	144 ± 29
Chronic hypertension (n = 18)			
AGA (n = 15)	929 ± 216	140 ± 30	135 ± 41
IUGR (n = 3)	950 ± 241	151 ± 30	154 ± 31

tween the variables, the probability of a single positive result in a patient depends on the number of assays (tests) performed, the definition of a positive antibody value, and the number of investigated patients. We consequently calculated the probability of one or more elevated antibodies, as expected for a binomial distribution when outcomes are equally likely.[17] In accordance, the expected number of patients to demonstrate one, two, three, and four elevated autoantibodies was 29%, 12.9%, 5.6%, and 2.4%, respectively, if all 42 isotypes were investigated. Consequently, only patients with five or more abnormally elevated autoantibodies were considered to demonstrate a significant B-cell activation. The same calculation revealed that three or more abnormal autoantibody elevations had to be considered significant if only one isotype (14 tests) was evaluated, three or more abnormalities were significant if only antiphospholipids were tested (18 tests), and two or more abnormalities if only antihistones and antipolynucleotides (12 tests each) were evaluated.

Contributing factors that may affect autoantibody production, such as age, gravidity, total immunoglobulin levels, methyldopa therapy, cigarette smoking, drug abuse, proteinuria, and systolic, diastolic, and mean blood pressure levels were included in a regression adjustment analysis. To evaluate whether the autoantibody profile was significantly different in the two hypertensive groups, a multivariant discriminant analysis was performed. The expected and actual number of autoantibodies and the incidence of birth weight under the 10th percentile were compared with the Fisher exact test. All statistical analyses were calculated with the Systat statistical computer package (Systat, Evanston, Ill.). All differences were assumed to be significant when $p < 0.05$ with two-tailed tests.

Results

Demographic data and laboratory profiles of all patients are described in Table I. Gestational ages in the various study groups did not differ significantly. The mean age of patients with chronic hypertension with AGA infants was higher than that of patients with mild as well as severe preeclampsia ($p < 0.05$) and normal control subjects ($p < 0.05$). The number of patients who smoked cigarettes or had a history of drug abuse was not statistically different between mothers who were delivered of AGA infants or infants with IUGR. All 18 patients with mild preeclampsia and six of 11 patients with severe preeclampsia were delivered of AGA infants. Five of 11 patients with severe preeclampsia and three of 18 patients with chronic hypertension were delivered of infants with IUGR. Preeclampsia was diagnosed in all three of these patients.

Proteinuria was significant only in those hypertensive patients delivered of infants with IUGR ($p < 0.05$). Platelet counts were significantly lower in patients with severe preeclampsia than those in normotensive patients and patients with mild preeclampsia ($p < 0.05$). Thrombocytopenia was observed in two of 11 patients with severe preeclampsia (one with and one without infants with IUGR) and in one of three patients with chronic hypertension who were delivered of an infant with IUGR. No significant differences were found in uric acid or creatinine values between hypertensive groups.

Total IgG, total IgM, and total IgA concentrations are presented in Table II. No significant differences were found between normotensive patients and the two hypertensive groups.

The number of elevated autoantibodies in hypertensive and control groups is presented in Fig. 1. Normotensive patients delivered of infants with IUGR demonstrated significantly more abnormally elevated antibodies than normotensive patients delivered of AGA infants ($p < 0.05$). However, even in the former group, only one patient exhibited more than five autoantibody abnormalities. In contrast, patients with preeclampsia and chronic hypertension, whether delivered of AGA infants or infants with IUGR, demonstrated a significant increase in autoantibody production over normotensive patients. It is more important, however, that within each hypertensive group, pregnancies that resulted in IUGR demonstrated significantly elevated autoantibody levels in comparison with pregnancies that resulted in AGA infants. No significant difference in autoantibody results was noted between patients with preeclampsia and chronic hy-

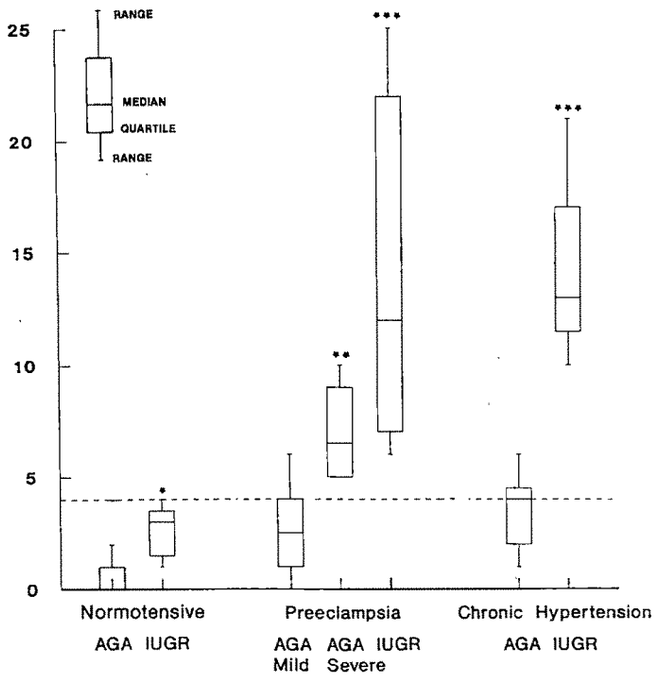


Fig. 1. Number of total autoantibodies (expressed by median and range) in normotensive and hypertensive groups. *One asterisk,* Significantly different from normotensive (AGA) group. *Two asterisks,* Significantly different from normotensive group (AGA and IUGR) and mild preeclampsia (AGA) ($p < 0.05$). *Three asterisks,* Significantly different from normotensive (AGA and IUGR) and hypertensive (AGA) groups ($p < 0.05$).

pertension who were delivered of AGA infants. Specific autoantibody abnormalities are shown in Fig. 2. The most frequently elevated autoantibodies were antiphospholipid, followed by antihistone and antipolynucleotide antibodies.

The highest number of elevated antibodies was found in patients with severe preeclampsia and chronic hypertension who were delivered of infants with IUGR. The most frequently observed antiphospholipid antibodies were antiphosphatidylglycerol, antiphosphatidylinositol, and antiphosphatidic acid antibodies. Only two of five patients with severe preeclampsia and one of three patients with chronic hypertension who were delivered of infants with IUGR demonstrated significantly elevated IgG anticardiolipin antibodies. In contrast, nine of 11 patients (82%) with severe preeclampsia and three of three patients (100%) with chronic hypertension who were delivered of infants with IUGR showed significantly elevated antibodies toward phosphatydilglycerol, phosphatydilinositol, and phosphatidic acid ($p < 0.05$). The highest incidence of antihistone antibodies was found in women with chronic hypertension, who were delivered of infants with IUGR and the most frequent antibodies observed were anti-H2A and anti-H3 subfractions. No significant antipolynucleotide abnormalities were observed in patients with either preeclampsia or chronic hypertension or in normotensive control patients.

An analysis of autoantibody isotypes (Fig. 3) indicates that IgG antibodies were most frequently elevated ($p < 0.05$). The highest incidence was found in hypertensive patients delivered of infants with IUGR. No significant differences in this regard were noted between the two hypertensive groups delivered of infants with IUGR. No correlation could be found between the levels of autoantibodies and the levels of total immunoglobulins IgG, IgM, and IgA.

Regression adjustment analysis of all previously noted potentially confounding variables excluded any of these parameters as contributing factors to the observed differences. Multivariant discriminant analysis indicated that the autoantibody profiles of hypertensive patients delivered of infants with IUGR were significantly different from both normotensive and hypertensive patients delivered of AGA infants (Wilks' Λ 0.11, $p < 0.01$).

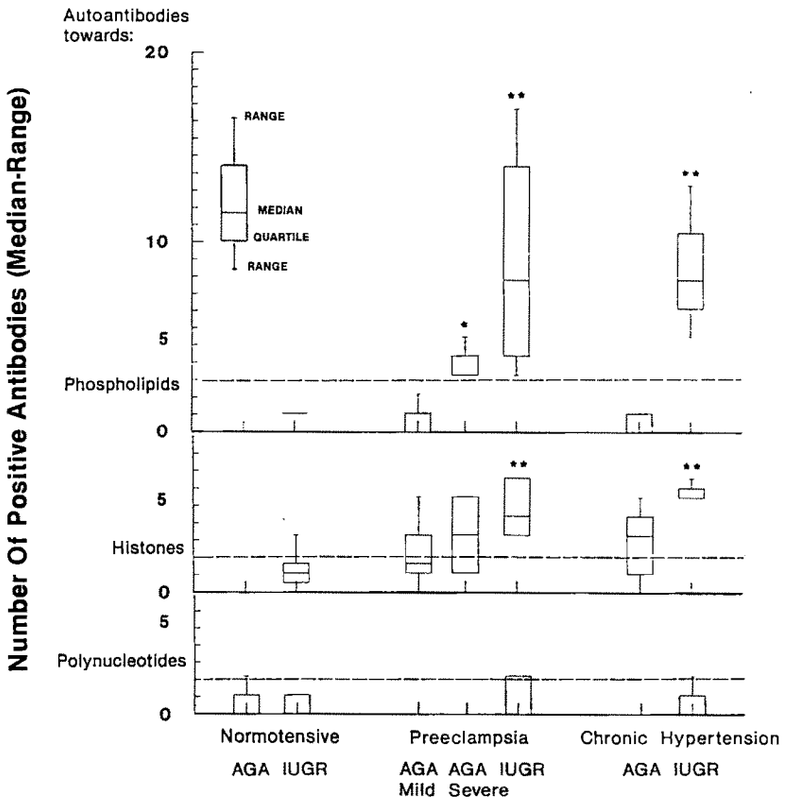


Fig. 2. Distribution of autoantibodies toward phospholipids, histones, and polynucleotides in normotensive and hypertensive groups. *One asterisk,* Significantly different from normotensive group (AGA and IUGR) ($p < 0.05$) and mild preeclampsia (AGA) ($p < 0.05$). *Two asterisks,* Significantly different from normotensive (AGA and IUGR) and hypertensive (AGA) groups ($p < 0.05$).

Comment

The purpose of this study was to examine the autoimmune response of patients with hypertensive disorders of pregnancy in relationship to the development of IUGR in offspring. As expected,[9, 10] IUGR occurred significantly more often in pregnancies with severe hypertension compared with patients with mild or no disease. Similarly, autoantibody production is related to severity of disease. It is important that mothers delivered of infants with IUGR demonstrated significantly more autoantibody production compared with mothers delivered of AGA infants in both hypertensive and normotensive groups.

One of the first observations to link IUGR and hypertensive disorders of pregnancy was made by McBurney[18] in 1947. Wildschut et al.[10] were able to demonstrate that multiparas with chronic hypertension had an increased incidence of IUGR compared with normotensive multiparas. Lin et al.[19] further expanded this observation by demonstrating the synergistic effect between hypertension and proteinuria in association with IUGR. In contrast to chronic hypertension, the relationship between pure preeclampsia and IUGR is less clear.[20] It appears that IUGR occurs in patients with preeclampsia only if the onset of disease is early and the disease is severe. Plouin et al.[21] observed that the onset of hypertension between 27 and 36 weeks' gestation was associated with the highest incidence of IUGR. Sibai[9] demonstrated a strong association between the severity of the disease, presence of proteinuria, and the appearance of IUGR.

Several studies have shown that patients with chronic hypertension and superimposed preeclampsia experience the highest incidence of IUGR. Wildschut et al.[10] reported that 42% of multiparas and 24% of nulliparas who had chronic hypertension with superimposed preeclampsia were delivered of infants with IUGR. Similar results were also reported by Sibai[9] and Gleicher et al.[22] Chesley[23] noted that patients with superimposed preeclampsia exhibited the worst perinatal outcome among all hypertensive patients. Our findings are consistent with a general pattern of increasing severity of disease, increasing incidence of IUGR, and increasing activation of the maternal immune system.

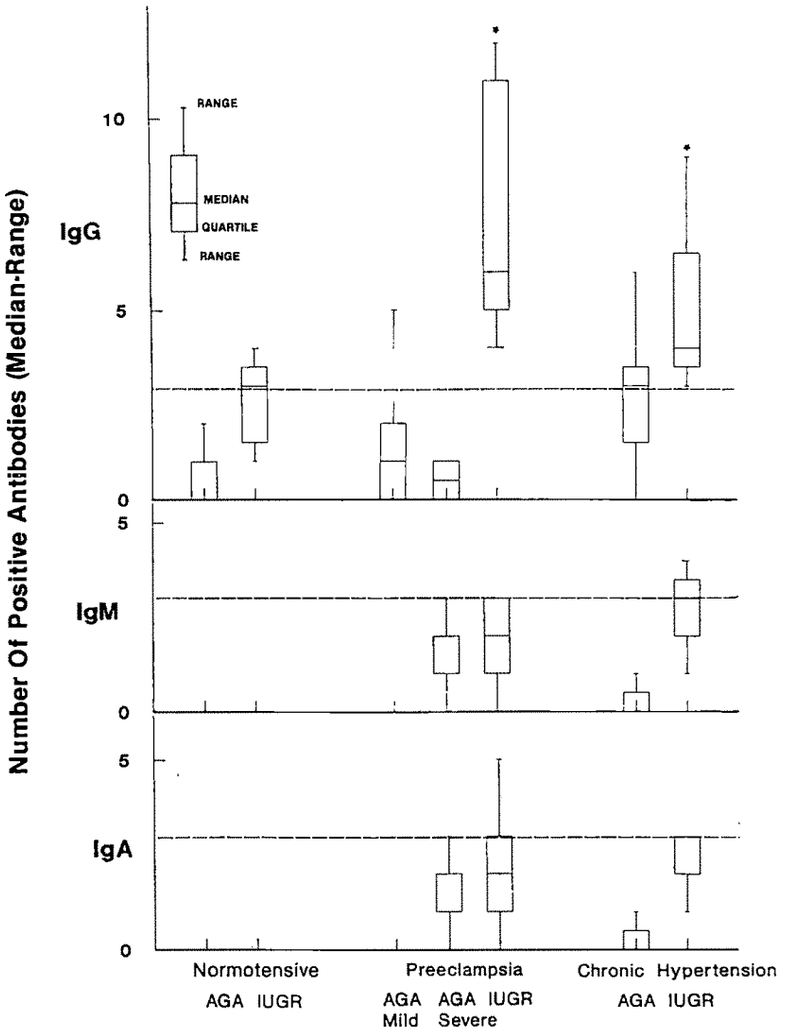


Fig. 3. Distribution of autoantibody isotypes in normotensive and hypertensive groups. *Asterisk*, Significantly different from normotensive (AGA and IUGR) and hypertensive (AGA) groups ($p < 0.05$).

Because the groups did not differ in the number of patients who smoked cigarettes or had a history of drug abuse, the mechanisms that cause diminished fetal growth are puzzling and raise some intriguing questions. Can the presence of abnormal autoantibodies contribute to the formation of vascular lesions in the placenta, which in turn compromise uteroplacental blood flow? Such lesions have been reported in placentas from patients delivered of IUGR infants.[24] The deposition of immune complexes,[25] complement components,[26] and the presence of chronic villitis in placentas of patients with hypertensive disorders,[27] autoimmune diseases,[28] and in placentas from idiopathic infants with IUGR[24, 27] lends support to such a concept.

Endothelial damage and the subsequent release of vasoactive compounds is recognized as part of the pathophysiology for some forms of hypertension in pregnancy.[29, 30] Thromboxane A_2 is released by vascular endothelium in the presence of lupus anticoagulant or antiphospholipid antibodies.[31] In this study, antiphospholipid antibodies were the most frequently recorded abnormalities in pregnancies, resulting in infants with IUGR. Those antibodies, and especially the IgG isotypes, have been associated with the presence of lupus anticoagulant and a predisposition toward arterial and venous thrombosis.[32] In this study antiphospholipid antibodies of the IgG isotype were most often produced and were demonstrated especially in pregnancies with IUGR. It is thus tempting to speculate that these autoantibodies may account for some of the placental damage seen in hypertensive disorders of pregnancy complicated by IUGR.

In pregnancies complicated by IUGR autoantibodies to phosphatidylglycerol, phosphatidylinositol, and phosphatidic acid occurred significantly more often than did anticardiolipin antibodies. Thus patients delivered of infants with IUGR either demonstrated a broad polyclonal activity or the production of specific clones that cross-reacted with different antigenic phospholipid determinants. Moreover, this finding questions the concentration by some investigators on anticardiolipin antibodies and lupus anticoagulant,[33, 34] a correlation that has also been questioned by others.[35-37] Thus these observations suggest that the production of antiphospholipid antibodies is not confined to a single autoantibody.

The highest incidence of antihistone antibodies was noted in patients with chronic hypertension and especially in those delivered of infants with IUGR. All the affected patients had methyldopa therapy. This drug is known to induce antihistone antibodies and, at times, even clinical symptoms consistent with systemic lupus erythematosus.[38] Whether the detection of antihistone antibodies in this study is drug-related or disease-related remains to be determined.

If autoantibody production can affect fetal growth, then suppression of this production may decrease the incidence of IUGR. Whereas clinical trials of immunosuppression have not been attempted so far, antithrombotic therapy, which can counteract the thrombotic effect of antiphospholipid antibodies, has recently been reported. Low-dose aspirin counteracts the excess production of thromboxane A_2[29] that may be induced by antiphospholipid antibodies.[31] Wallenburg et al.[39] used aspirin and dipyridamole to treat normotensive pregnant women who previously were delivered of infants with idiopathic IUGR. Recurrent fetal growth retardation occurred in 13% of treated and 61% of untreated pregnancies. The same author[40] also reported that a similar low-dose aspirin protocol caused a decrease in the subsequent occurrence of maternal preeclampsia. These observations suggest that treatment directed at the effect of abnormal B-cell activation may be useful to prevent IUGR and may have benefit in the treatment of hypertensive disorders of pregnancy.

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Umbilical arterial and venous acid-base and blood gas values and the effect of chorioamnionitis on those values in a cohort of preterm infants

Gary D.V. Hankins, Lt Col, USAF, MC, Russell R. Snyder, Lt Col, USAF, MC, and Edward R. Yeomans, Maj, USAF, MC

San Antonio, Texas

Umbilical arterial and venous acid-base and blood gas values in uncomplicated premature births are similar to values that are reported in term infants and are unaffected by birth weight or gestational age. In this group of patients chorioamnionitis had no significant effects on umbilical arterial acid-base or blood gas values or on the percentage of patients that were born with acidemia. Apgar scores were significantly lower in the group with chorioamnionitis in spite of a virtual absence of acidemia, which again suggests that low Apgar scores alone do not confirm a diagnosis of birth asphyxia. (AM J OBSTET GYNECOL 1991;164:1261-4.)

Key words: Apgar scores, chorioamnionitis, cord acid-base status, preterm

Assessment of acid-base status by means of umbilical cord blood sampling provides an objective method of evaluation of either fetal or newborn condition.[1, 2] In contrast, the Apgar score is a very subjective measurement, which may vary substantially depending on the skill, experience, and bias of the individual who assigns the score. Further, in regard to the preterm infant, a low Apgar score has not been shown to be predictive of acidemia or of asphyxia.[3]

To date, there is a paucity of information on umbilical arterial acid-base and blood gas determinations in preterm infants and an absence of published information about umbilical venous blood values. Ramin et al.[4] found no significant differences in umbilical arterial acid-base or blood gas values in preterm infants compared with term infants. They also found no significant

From the Department of Obstetrics and Gynecology, Wilford Hall United States Air Force Medical Center, Lackland Air Force Base.
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Reprint requests: Gary D.V. Hankins, MD, Wilford Hall USAF Medical Center/SGHO, Lackland AFB, TX 78236-5300.
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Table I. Umbilical arterial acid-base and blood gas determinations (mean ± SD) stratified by birth weight

	<1499 gm (n = 12)	*1500 to 1999 gm (n = 20)*	*2000 to 2499 gm (n = 54)*	*Total (N = 86)*
pH	7.31 ± 0.07	7.27 ± 0.07	7.27 ± 0.07	7.28 ± 0.07
P_{CO_2}	44.98 ± 7.2	47.36 ± 7.42	48.84 ± 10.3	47.96 ± 9.41
P_{O_2}	21.78 ± 6.24	18.39 ± 6.6	19.35 ± 7.08	19.47 ± 6.93
Bicarbonate	22.24 ± 3.06	21.76 ± 3.51	22.11 ± 2.64	22.04 ± 2.93
Base excess	−2.37 ± 2.48	−4.58 ± 3.95	−4.13 ± 2.95	−4.06 ± 3.26

differences when preterm infants were stratified according to birth weight.

The purpose of this investigation was to provide additional information regarding umbilical arterial values and to report umbilical venous values in infants with low birth weight. Additionally, we sought to determine the effects of chorioamnionitis on the newborn's immediate metabolic condition as reflected by umbilical cord pH and blood gas values and to compare this information to that provided by Apgar scores.

Patients and methods

Woman and infant pairs that were included in this study were delivered at Wilford Hall United States Air Force Medical Center between October 1984 and April of 1990. The study population consisted of all women whose fetuses weighed <2500 gm at birth, were <37 weeks' gestational age, and for whom umbilical cord blood pH and blood gas determinations were available. All complications of pregnancy except premature labor with or without rupture of fetal membranes and/or chorioamnionitis were excluded from analysis. Clinical diagnosis of chorioamnionitis consisted of maternal fever and uterine tenderness or uterine tenderness and concomitant fetal tachycardia. Although the latter criteria were sufficient to make the initial clinical diagnosis and to institute antibiotic therapy, it was noted that in each instance these women had fevers before delivery. All cases of preeclampsia, intrauterine growth retardation, and fetal distress were excluded. Specifically, fetal distress that was sufficient to warrant exclusion from analysis consisted of a fetal heart rate pattern that was of enough concern to result in either an operative vaginal delivery or a cesarean delivery. Umbilical cord prolapse, which is uniformly treated with cesarean delivery, also was categorized as fetal distress. The antepartum, intrapartum, and postpartum records of each of these women were carefully reviewed to complete the data base.

Among the 110 women-infant pairs, 24 had chorioamnionitis and 86 had no complications except for premature labor and/or premature rupture of membranes. The latter group was managed expectantly in the absence of signs or symptoms of infection or fetal distress. The 86 infants without chorioamnionitis were subdivided, on the basis of birth weight, into categories of ≤1499, 1500 to 1999, and 2000 to 2499 gm. In all cases gestational age was well established by a combination of menstrual data, obstetric examination, and ultrasonographic evaluation.

At our institution, umbilical arterial and venous pH and blood gas determinations are routinely performed at all deliveries, as previously described.[2] All specimens are analyzed within 5 minutes of collection on an ABL-2 analyzer (Radiometer, Copenhagen), which is maintained in the neonatal intensive care unit. Two blood gas analyzers operate continuously and are calibrated against known standards several times daily. They are also autocalibrated before each individual blood analysis. Fetal acidemia was defined as an umbilical arterial pH <7.20 with further stratification to pH values <7.10 and <7.0 to more stringently define acidemia.

Apgar scores were assigned by the pediatricians in attendance at each of the deliveries. Five infants in the group with chorioamnionitis and seven in the group without evidence of infection were not assigned Apgar scores because of early intubation and its effects on the Apgar score.

Statistical significance was determined by means of the two-tailed t test, χ^2 analysis, or the Fisher exact probability test, as appropriate. Significant differences were determined at $p < 0.05$.

Results

The umbilical arterial and venous acid-base values, which were stratified by birth weight, are shown in Tables I and II, respectively, for those infants with no evidence of chorioamnionitis. No significant differences existed for any blood gas or acid-base parameter relative to birth weight. An absolute pH <7.20 was noted in one of 12 infants who weighed <1499 gm, three of 20 infants who weighed 1500 to 1999 gm, and seven of 54 infants who weighed 2000 to 2499 gm, and there was no significant difference for any birth weight category.

Comparison of acid-base and blood gas parameters from uncomplicated term deliveries, as previously reported by our institution,[2] and of values in preterm deliveries with and without chorioamnionitis can be found in Table III. The only significant difference that

Table II. Umbilical venous acid-base and blood gas determinations (mean ± SD) stratified by birth weight

	<1499 gm (n = 12)	*1500 to 1999 gm (n = 20)*	*2600 to 2499 gm (n = 54)*	*Total <2499 gm (N = 86)*
pH	7.36 ± 0.07	7.34 ± 0.08	7.33 ± 0.05	7.34 ± 0.06
P_{CO_2}	38.55 ± 6.02	39.0 ± 7.5	41.23 ± 5.8	40.34 ± 6.38
P_{O_2}	30.53 ± 7.74	27.12 ± 6.78	27.09 ± 7.56	27.58 ± 7.50
Bicarbonate	21.63 ± 1.52	20.98 ± 3.08	21.28 ± 2.09	21.25 ± 2.32
Base excess	−3.56 ± 3.13	−4.57 ± 3.58	−3.92 ± 2.55	−4.04 ± 2.92

Table III. Comparison of acid-base and blood gas values for uncomplicated term deliveries, premature deliveries without other complications, and premature deliveries with chorioamnionitis

Infants	*N*	*pH*	*P_{CO_2}*	*P_{O_2}*	*Bicarbonate*	*Base excess*
Arterial						
Term	146	7.28 ± 0.05	49.2 ± 8.4	18.0 ± 6.2	22.3 ± 2.5	—
Preterm	86	7.28 ± 0.07	47.96 ± 9.41	19.47 ± 6.93	22.04 ± 2.93	−4.06 ± 3.26
Preterm-chorioamnionitis	24	7.26 ± 0.10	48.71 ± 14.29	17.98 ± 7.06	21.07 ± 3.75	−5.29 ± 4.54
Venous						
Term	146	7.35 ± 0.05	38.2 ± 5.6	29.2 ± 5.9	20.4 ± 2.1	—
Preterm	86	7.34 ± 0.06	40.34 ± 6.38	27.58 ± 7.5	21.25 ± 2.32	−4.04 ± 2.92
Preterm-chorioamnionitis	24	7.32 ± 0.11	41.19 ± 13.6	29.7 ± 9.21	19.9 ± 4.06*	−5.63 ± 4.48

Data are presented as mean ± SD.
*$p = 0.044$, preterm versus preterm-chorioamnionitis.

was noted was a lower bicarbonate value in the umbilical venous blood of the preterm infants with chorioamnionitis versus those that were uninfected ($p = 0.044$). With respect to pH values <7.20, four of 24 (16.7%) infants with chorioamnionitis and 10 of 86 (11.6%) infants without infection had this degree of acidemia (p = NS). More severe acidemia was rare; a pH <7.10 was noted in two of 24 infants with chorioamnionitis versus one of 86 uninfected infants. A pH <7.0 was noted in one infected infant and in one uninfected infant.

Maternal demographics and puerperal variables were analyzed according to the presence or absence of chorioamnionitis. No significant differences existed for maternal age, route of delivery, breech versus vertex presentation, length of the first or second stage of labor, or percent of infants that required endotracheal intubation at delivery. Those neonates whose courses were complicated by chorioamnionitis were significantly younger (209.5 ± 21.1 vs 235.8 ± 19.8 days, mean ± SD, $p < 0.00001$) and correspondingly much smaller (1562 ± 502 vs 2018 ± 437 gm, mean ± SD, $p < 0.00001$) than those that were born prematurely but without chorioamnionitis. Additionally, as a group the mean Apgar score at 1 minute was depressed relative to that of those infants without infection (5.3 ± 2.8 vs 6.9 ± 2.0, mean ± SD, $p < 0.006$). Infants with chorioamnionitis also had a higher percentage of Apgar scores <3 at 1 minute (5/19 vs 5/79, $p < 0.01$) and <7 at 5 minutes (5/19 vs 8/79, $p < 0.05$) than did their counterparts who were not infected. The mean Apgar score at 5 minutes was 7.6 ± 2.0 in infants with chorioamnionitis and 8.0 ± 1.7 in those without infection (p = NS).

Comment

The objective of this investigation was to establish normative data for a population of patients with otherwise uncomplicated premature labor and delivery and to compare our results with data that have been previously published. Umbilical arterial acid-base and blood gas parameters in the population reported here are very similar to those reported by Ramin et al.[1] for uncomplicated premature deliveries. In accordance with those investigators, we also found no statistically significant effects on the basis of birth weight, or by extrapolation for gestational age, on any parameter measured.

We also report umbilical venous acid-base and blood gas values for 86 preterm deliveries, which were otherwise uncomplicated. Birth weight, as well as arterial specimens, had no significant effect on any parameter that was measured. Further, comparison of both the arterial and venous values for uncomplicated term deliveries, as previously reported,[2] reveals no effect of gestational age on acid-base or blood gas values.

The second major objective of this study was to quantify the effect of chorioamnionitis in the premature infant on the immediate metabolic condition at birth. Although the umbilical arterial pH was somewhat lower (7.26 vs 7.28) in those infants with chorioamnionitis and the base excess was somewhat higher (−5.29 vs −4.06)

than in the preterm infants without infection, neither value achieved statistical significance. A similar trend was seen in the umbilical venous specimens in regard to pH and base excess; however, in this instance the concentration of bicarbonate was lower ($p = 0.044$). With the use of absolute pH values to define acidemia, we again found no significant difference in those infants with chorioamnionitis relative to those who were not infected. Significantly, factors that might have influenced these values (such as length of labor, abnormal presentations, or operative abdominal or vaginal delivery) were not significantly different between those with and those without chorioamnionitis. The lower birth weights and less advanced gestational ages, which are both highly significant in infants who were infected relative to infants who were not infected, also cannot independently account for any of the differences. However, infection did appear to result in earlier delivery as compared with that in the group without an identifiable precipitant of the labor and/or amniorrhexis.

If Apgar scores had been used to predict metabolic status, they would have predicted a much greater effect in those infants with chorioamnionitis than was shown to exist by acid-base assessment. The analysis of low Apgar scores excluded those infants who were intubated by a pediatrician at birth. This exclusion was elected because in many instances the intubation was performed prophylactically and purely on the basis of gestational age. Reanalysis to include low Apgar scores and those infants who were intubated did not alter the results, statistical significance, or conclusions.

The Apgar score alone has been shown to be an unreliable predictor of neonatal acidemia and asphyxia in the preterm infant.[3, 5] Apgar scores have also been judged insufficient in and of themselves to define asphyxia in term infants.[1, 6, 7] In addition, Mayberry et al.[8] have shown that significantly more infants with chorioamnionitis have low 1-minute (20% vs 5%) and 5-minute (3% vs 1%) Apgar scores than do infants without infection. The population that was reported on by these investigators consisted primarily of term gestations. Current data further suggest the inadequacy of the Apgar score as a marker of birth asphyxia in preterm infants with chorioamnionitis since depressed Apgar scores in this group were seldom associated with umbilical arterial acidemia.

In conclusion, umbilical arterial acid-base and blood gas values in the cohort of preterm infants that are reported on here are similar to those reported on by other investigators.[4] Additionally, they are not significantly different from values reported in uncomplicated term deliveries[5] and are not affected by birth weight or by the extrapolated gestational age. Chorioamnionitis in the preterm infant did result in significant depression of Apgar scores but had no effect on acid-base or blood gas values and did not lead to acidemia. Accordingly, the diagnosis of birth asphyxia in the preterm infant, with or without chorioamnionitis, requires cord blood analysis; the Apgar score alone is not sufficient.

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Cervical human papillomavirus deoxyribonucleic acid and cytologic evaluations in gynecologic outpatients

Pekka Nieminen, MD,[a] Valeria R.X. Soares, MSc,[a] Markku Aho, MD,[a] Ervo Vesterinen, MD,[a] Eeva Savia, MD,[a] Antti Vaheri, MC,[b] and Jorma Paavonen, MD[a]
Helsinki, Finland

We studied the prevalence and cytologic manifestations of human papillomavirus deoxyribonucleic acid among 2668 gynecologic outpatients. The study population consisted of 1573 women who came into the emergency room, 623 women from an abortion clinic who were seen because of induced first-trimester abortion, and 472 women who had been referred to a colposcopy clinic because of an abnormal Papanicolaou smear finding. A dot blot technique was used for the detection of human papillomavirus types 6/11, 16/18, and 31/33/35. The prevalence of human papillomavirus deoxyribonucleic acid was 4.4% in the emergency room population, 8.8% in the abortion clinic population, and 31.4% in the colposcopy clinic population. Human papillomavirus type 16/18 was most prevalent (3.5%), followed by human papillomavirus type 31/33/35 (3.1%), and human papillomavirus type 6/11 was least prevalent (2.1%). Of all unselected human papillomavirus deoxyribonucleic acid–positive women who were enrolled from the emergency room or from the abortion clinic, 73% had normal cytologic evaluations. Twenty-five percent had benign atypia, and 2% had dyskaryotic changes. No human papillomavirus deoxyribonucleic acid type-specific differences were detected in the distribution of cytologic atypias. (Am J Obstet Gynecol 1991;164:1265-9.)

Key words: Cervical HPV DNA, cytology

There is increasing interest in the epidemiology of human papillomavirus (HPV) infection in unselected populations. Cytologic studies that are based on the detection of koilocytes in cervical cell samples suggest relatively low prevalence, whereas studies that utilize HPV deoxyribonucleic acid (DNA) hybridization techniques or polymerase chain reaction suggest high prevalence of HPV DNA both among selected and unselected women.[1-11] The prevalence of HPV DNA seems to vary greatly depending on the detection technique used, the number of HPV types that are studied, the type of population that is studied, and the geographic origin of the study population. Furthermore, interlaboratory variation in the prevalence estimates of HPV DNA has probably been underestimated.

Although the presence of HPV DNA does not necessarily indicate an active papillomavirus infection, it is important to learn more about the epidemiologic correlates of HPV DNA in order to develop clinical management strategies. This study was undertaken to determine the overall prevalence of HPV DNA, the prevalence of selected HPV DNA types, and cytologic correlates of HPV DNA among women who were seen in a metropolitan gynecologic outpatient clinic. On the basis of the screening results a study was set up, in which women, who were primarily HPV DNA-positive but had normal cervical cytologic evaluations, were followed up. These results are to be reported later.

From the Department of Obstetrics and Gynecology, University Central Hospital Helsinki,[a] and the Department of Virology, University of Helsinki.[b]
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Reprint requests: Pekka Nieminen, MD, Department of Obstetrics and Gynecology, University Central Hospital, Haartmaninkatu 2, 00290 Helsinki, Finland.
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Patients and methods

Study population. The study was conducted at the Department of Obstetrics and Gynecology at University Central Hospital in Helsinki, Finland, between February 1988 and December 1988. The study population consisted of 1573 consecutive women who came into the emergency room of the outpatient clinic for the first time, 623 consecutive women who were seen because of induced first-trimester abortion, and 472 consecutive women who were referred to a colposcopy clinic because of an abnormal cervicovaginal cytologic (Papanicolaou) smear. We excluded women who had undergone hysterectomy, who had taken antibiotics during the previous 2 weeks, or who had been examined for the same problem in the same clinic within the last month.

Patient evaluation. All women underwent a standard gynecologic examination. Cervicovaginal cyto-

Table I. Selected demographic, historic, and microbiologic characteristics of the study populations

Characteristics	Groups					
	Emergency room		*Abortion clinic*		*Colposcopy clinic*	
	HPV+	*HPV−*	*HPV+*	*HPV−*	*HPV+*	*HPV−*
Age* (yr)	28.3 (16-61)	30.4 (16-52)	23.1 (17-38)	28.0 (15-47)	25.3 (16-54)	28.6 (18-65)
Menarche (yr)	13.1	13.7	12.7	12.9	13.3	13.5
Gravidity*	1.4 (0-12)	1.8 (0-8)	1.9 (1-14)	2.3 (1-11)	0.5 (0-5)	0.9 (0-5)
Parity*	0.6 (0-3)	1.0 (0-3)	0.3 (0-2)	0.8 (0-5)	0.3 (0-3)	0.6 (0-5)
Contraception (%)						
Oral contraceptives	33	31	NA	NA	52	50
Intrauterine contraceptive device	6	7			7	10
Condom	11	17			15	14
None	34	39			9	25
Other	16	6			17	1
Currently smoking (%)	49	33	53	46	35	38
No. of lifetime partners*	7 (1-30)	5 (1-10)	13 (1->100)	Unknown	7 (1-40)	6 (1-50)
Age at first intercourse (yr)	17	17	16	Unknown	17	17
History of STDs† (%)	37‡	17	62‡	19	54§	45

HPV, Human papillomavirus; *NA*, not applicable; *STDs*, sexually transmitted diseases.

*Mean and range.

†Sexually transmitted diseases include *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, herpes simplex virus, *Trichomonas vaginalis*, and condyloma.

‡$p < 0.001$.

§Not significant.

logic smear (Papanicolaou smear) was first taken with an Ayres spatula and by swabbing the endocervical canal with the Cytobrush (Medscand AB, Malmö) cervical sampler. The Papanicolaou smears were stained with modified Papanicolaou stain and reviewed by one cytologist who had no knowledge of the clinical or laboratory findings. The cytologic diagnosis of koilocytosis was based on the presence of characteristic "halo" cells with cytoplasmic perinuclear clearing.[12] The presence of dyskeratosis, binucleation, parakeratosis, or other mild cytologic abnormalities (benign atypias that were not consistent with dysplasia) was also noted.[13] When dyskaryotic changes were noted, the smears were interpreted as suggestive of dysplasia regardless of the presence or absence of koilocytosis.[14] Dyskaryotic smears were further classified as suggestive of mild, moderate, or severe dysplasia, or of carcinoma in situ according to standard criteria.[15]

Detection and typing of HPV DNA. Samples for the detection of HPV DNA were taken from the endocervix and from the transformation zone with a cotton-tipped swab and placed into specimen transport media (ViraPap specimen collection kit, Life Technologies Inc., Gaithesburg, Md.). Phosphorus 32–labeled ribonucleic acid probes for HPV types 6, 11, 16, 18, 31, 33, and 35 were used for detection of HPV DNA by dot blot hybridization (Virapap and Viratype, Life Technologies Inc.). The Virapap test detects HPV types 6, 11, 16, 18, 31, 33, and 35 as a cocktail, and the Viratype test distinguishes HPV types 6 or 11, 16 or 18, and 31, 33, or 35 as three different HPV DNA groups. The samples were first incubated with sample preparation reagent for 1 hour at 37° C. After cell lysis, 250 μl was removed and transferred to new tubes. These samples were treated with sample diluent (15 minutes at room temperature) to achieve DNA denaturation, applied to a presoaked nylon membrane in a 25-well ViraPap filter manifold (Life Technologies Inc.) and filtered under vacuum. Thereafter the filter was transferred to a reaction tray, and 5 ml of prewarmed prehybridization reagent was added. After prehybridization in a water bath (60° C) for 30 minutes, membrane-bound HPV-target DNA was hybridized to full-length ribonucleic acid transcripts that are homologous to HPV genotypes 6, 11, 16, 18, 31, 33, and 35 under stringent conditions (60° C for 2 hours). After three washes, the membranes were treated with ribonuclease for 15 minutes at 37° C to remove unhybridized probe and again washed three times at 60° C under highly stringent conditions. Bound probe was detected by autoradiographic exposure to Kodak X-Omat AR x-ray film (Eastman Kodak Co., Rochester, N.Y.) with intensifying screens at −70° C. The exposure time varied from 1 to 3 days. The signal was interpreted by comparison to negative controls, low-positive controls, and high-positive controls. The specimen was considered positive if the intensity of the autoradiographic signal was stronger than that of the low-positive control (Fig. 1). All autoradiographs were independently interpreted by three of the authors. The interobserver agreement was 97%. Discordant results

Table II. Prevalence of HPV types in the three study groups

	Group						*Total (N = 2668)*	
	Emergency room (n = 1573)		*Abortion clinic (n = 623)*		*Colposcopy clinic (n = 472)*			
HPV DNA types	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
6/11	11	0.7	6	1.0	39	8.3	56	2.1
16/18	26	1.7	27	4.3	41	8.7	94	3.5
31/33/35	25	1.6	18	2.6	41	8.7	84	3.1
Mixed*	7	0.4	4	0.8	27	5.7	38	1.5
TOTAL	69	4.4	55	8.8	148	31.4	272	10.2

*Two or more of the three HPV DNA groups.

Table III. Correlation of HPV genotypes with cytologic findings among unselected women from the emergency room and from the abortion clinic

	HPV DNA positive										*HPV DNA negative (N = 228)*	
	6/11 (n = 11)		*16/18 (n = 48)*		*31/33/35 (n = 34)*		*Mixed (n = 9)*		*Total (N = 102)*			
Cytologic findings	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Normal	9	82	33	69	26	76	6	67	74	73	208	92
Benign atypia	2	18	13	27	8	24	3	33	26	25	16	6
Dyskaryosis	0		2	4	0		0		2	2	4	2

were discussed by the three authors until consensus was reached. The ViraType HPV DNA typing kit (Life Technologies Inc.) applied the same procedures as those that were described for the ViraPap kit, with the following exceptions. After cell lysis, 450 μl was removed and treated with sample diluent to achieve DNA denaturation. The samples were then applied in equal aliquots to three nylon membranes and filtered under vacuum. After prehybridization, the membranes were hybridized with three separate probe mixes specific for HPV types 6/11, 16/18, or 31/33/35. The hybridization conditions, all remaining wash steps, and autoradiography were the same as those described for the ViraPap test.

The limits of detection of HPV DNA with ViraPap and ViraType tests are approximately 3 pg for ViraPap and 1 pg for ViraType.[16]

Statistical methods. Comparisons between the study groups with and without HPV DNA were performed either by the χ^2 test for proportions or Student's *t* test for means.

Results

Demographic characteristics. Selected demographic characteristics of the study populations are shown in Table I. HPV DNA-positive women were younger, had lower parity, and more often had a history of sexually transmitted diseases than HPV DNA-negative women. Number of lifetime sexual partners was also higher, but the difference was not statistically significant. There were no differences in contraceptive methods, smoking habits, or age at the first intercourse between the two groups. However, no differences were observed among the three study populations—emergency room, abortion clinic, and colposcopy clinic—in any of these parameters, except in the history of sexually transmitted diseases (Table I).

Type-specific prevalence of HPV DNA. The prevalence of HPV DNA was 4.4% in the emergency room clinic population, 8.8% in the abortion clinic population, and 31.4% in the colposcopy clinic population (Table II). The prevalence of HPV 16/18 was somewhat higher (3.5%) than that of HPV 31/33/35 (3.1%) or HPV 6/11 (2.1%). Thirty-eight patients (1.4%) tested positive for more than one of the three HPV groups, which represented 14.0% of all DNA-positive findings. Less than 1% of the women who were seen in the emergency room or in the abortion clinic had macroscopically visible genital warts, whereas 15% of the women who were seen in the colposcopy clinic had visible genital warts.

Cytologic correlates of HPV DNA. We also analyzed cytologic findings in relation to genital HPV infection. Simultaneously taken HPV DNA samples and cytologic

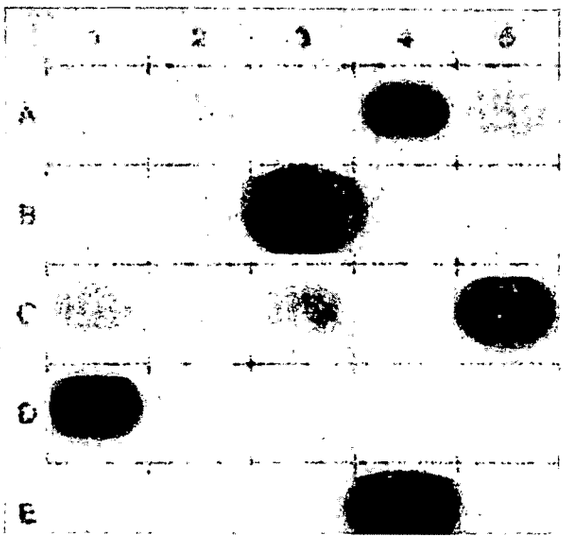


Fig. 1. Autoradiograph from a ViraPap test. *Row A, column 3* is "negative control," *A4* is "high-positive" control, and *A5* is "low-positive" control. Sample intensities below low-positive control (e.g., *A2*) are interpreted to be HPV DNA negative.

smears were available from 236 of 272 HPV DNA-positive women, and the control group consisted of 228 consecutive HPV DNA-negative women who were seen in the emergency room or in the abortion clinic. Among unselected women (emergency room and abortion clinic populations) (Table III) who tested positive for HPV DNA, 73% (74/102) had normal cytologic evaluations, 25% (26/102) had benign atypia, and 2% (2/102) had dyskaryosis. In the HPV DNA-negative group 92% (208/228) had normal cytologic evaluations, 2% (4/228) had dyskaryosis, and the remaining 6% (16 of 228) had benign atypia. Thus any atypia was present in 27% of the HPV DNA-positive women and in 8% of the HPV DNA-negative women. The difference in the overall distribution of cytologic findings between HPV DNA-positive and HPV DNA-negative women was statistically significant ($p < 0.001$). When the cytologic findings were correlated with specific HPV types, no significant differences were observed (Table III).

Women who were enrolled from the colposcopy clinic ($n = 472$) had, as expected, clearly more cytologic abnormalities (71%). In this group HPV type 16/18 and HPV type 31/33/35 were more often associated with dyskaryosis than was HPV type 6/11, but the differences were not significant (33% and 38% vs 25%, respectively) (data not shown).

Comment

We studied the prevalence of cervical HPV DNA by dot blot hybridization in a large number of women who visited a gynecologic outpatient clinic of a major university hospital in Helsinki, Finland. The prevalence of HPV DNA was 4.4% among unselected women who came into the emergency room, 8.8% among unselected women who were seeking induced abortions, and 31.4% among women who were referred to the colposcopy clinic because of an abnormal finding in a Papanicolaou smear. In earlier reports the prevalence of cervical HPV DNA has varied from 0.5% to 35%, although direct comparison among different studies is difficult because of differences in the hybridization techniques that were used.[11, 17] In many studies, however, only a limited number of HPV types (usually 6, 11, 16, and 18) have been studied, and the study populations have generally been more or less selected. HPV DNA has been detected (by Southern blot, dot blot, or in situ hybridization) in 30% to 91% of nonpregnant women who have cytologic evidence of cervical intraepithelial neoplasia and in 1% to 13% of nonpregnant women who have normal cytologic evaluations. In this study the prevalence of HPV DNA in unselected women from the emergency room was relatively low as compared with previous reports. However, among women from the abortion clinic, the prevalence was clearly higher, which is not surprising since these women had more lifetime sex partners and more often had a history of sexually transmitted diseases, although they were younger. No differences were observed between HPV DNA-positive and HPV DNA-negative women in parity, method of contraception, or smoking, which is somewhat surprising since previous studies have suggested that smoking and use of oral contraceptives are risk factors for HPV infection and cervical intraepithelial neoplasia.[18, 19] However, adjustments for duration and frequency of smoking or duration of use of oral contraceptives might have revealed significant differences between the groups.

Overall, only 14% to 20% of all positive HPV DNA findings were types 6 and 11. HPV types 16 and 18 were most prevalent and accounted for almost 50% of all unselected HPV DNA-positive women. Kiviat et al.[8] and Lorincz et al.[20] have reported similar findings in previous studies. This suggests that HPV types 16 and 18 are the most prevalent HPV types among individuals who do not have exophytic condylomas. We do not know whether these women are at increased risk for cervical neoplasia or how they should be managed. The high frequency of HPV types 16 and 18 DNA in unselected women and the decreasing incidence of cervical cancer in industrialized countries suggest that the risk of invasive cancer to individuals with HPV type 16 or HPV type 18 DNA is very low.

Although there is a tendency to assume that HPV DNA indicates HPV infection, this is not necessarily true. It is more likely that in many cases HPV DNA merely reflects the presence of virus or viral particles and not active infection. Before definitive conclusions from the link between HPV DNA and cervical cancer solely on the basis of the presence of HPV DNA can

be drawn, more studies of the transmission and natural history of HPV DNA are needed.

We found that nearly three fourths of the unselected HPV DNA-positive women had normal cytologic evaluations, and of all women with normal cytologic evaluations 4.5% tested positive for HPV DNA. In previous studies among women with normal cytologic findings, HPV DNA has been detected in 1% to 13% of subjects.[17] Although cytologic atypias (benign atypias and dyskaryosis) were more common among HPV DNA-positive women than among HPV DNA-negative women, no significant differences were seen among different HPV DNA types. These results differ from earlier reports, which have consistently shown that HPV types 16 and 18 are associated with dyskaryosis.[2, 3, 5, 20-24] One explanation is that in our study only few women had changes that were consistent with cervical epithelial neoplasia, and in fact, none had changes that were consistent with cervical epithelial neoplasia 2 or 3. HPV types 16 and 18 are more strongly associated with cervical epithelial neoplasia 2 to 3 than with cervical epithelial neoplasia 1.[25] Furthermore, although the prevalence of HPV types 16 and 18 and HPV types 31, 33, and 35 is commonly higher than that of HPV types 6 and 11, it is obviously higher also among women with cervical epithelial neoplasia. This might partly affect the interpretation of results, if the study population is selected on the basis of existing cervical epithelial neoplasia.

Previous HPV prevalence studies and annual incidence rates of cervical cancer suggest that up to 3% of HPV-positive women will have cervical cancer in 20 to 50 years.[7, 21, 26, 27] Natural history studies that utilize cytologic and colposcopic findings among women who are free of symptoms are also needed to determine cofactors that are of importance in the development of cervical neoplasia among women with or without cervical HPV DNA.

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A prospective genetic study of complete and partial hydatidiform moles

Sylvia D. Lawler, MD,[a] Rosemary A. Fisher, PhD,[b] and Joan Dent, SRN[b]
London, England

Two hundred two hydatidiform moles were classified by pathologic features and ploidy into partial or complete moles. Further classification was made by using genetic polymorphisms, the diagnosis being refined by deoxyribonucleic acid technology. Among 51 partial hydatidiform moles, 44 triploids, two tetraploids, and one diploid were identified. Informative triploid partial hydatidiform moles had one maternal and two paternal sets of chromosomes, the likely origin being dispermy. Among 149 complete hydatidiform moles, one was haploid, one was triploid, and 105 were shown to be diploid or androgenetic; 39 of these were proved homozygous, indicating duplication of the male genome, whereas heterozygous origin by dispermy was likely in 13. The locus-specific minisatellite deoxyribonucleic acid probes were particularly useful for the identification of heterozygous complete hydatidiform moles. None of the patients with partial hydatidiform moles had development of a gestational trophoblastic tumor. No difference was detected in the frequency of requirement for chemotherapy between patients with homozygous or heterozygous complete hydatidiform moles. (AM J OBSTET GYNECOL 1991;164:1270-7.)

Key words: Hydatidiform mole, gestational trophoblastic tumor, genetics

Hydatidiform moles are recognized macroscopically and classified by histopathologic examination into two categories, partial and complete.[1]

Genetic studies have established that partial hydatidiform moles are usually triploid, having two paternal and one maternal set of chromosomes,[2] whereas complete hydatidiform moles are diploid and androgenetic, having two paternal chromosome sets.[3] The majority of complete moles are genetically homozygous, having arisen by duplication of a haploid sperm genome, whereas a minority arise by dispermy and are therefore heterozygous at some loci.[4]

In both partial and complete hydatidiform moles the mitochondrial deoxyribonucleic acid (DNA) is maternal in origin as in a normal conceptus.[4] We describe a prospective study carried out between 1978 and 1987 in which hydatidiform moles were classified according to pathologic features, ploidy, and genetic origin. Follow-up of the patients enabled us to relate the type of hydatidiform mole to the risk of development of a gestational trophoblastic tumor.

From the Institute of Cancer Research, Chester Beatty Laboratories,[a] and the Cancer Research Campaign Laboratories, Department of Medical Oncology, Charing Cross and Westminster Medical School.[b]
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Reprint requests: Professor S.D. Lawler, Institute of Cancer Research, Chester Beatty Laboratories, Fulham Road, London SW3 6JB, United Kingdom.
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Patients and methods

Patients. Fresh tissue and, where possible, 10 ml of heparinized blood from the patient and her partner were collected at termination of pregnancy for a suspected hydatidiform mole.

Pathologic diagnosis. Two hundred forty-nine samples of tissue were collected from participating hospitals, examined macroscopically and histopathologically, and classified as nonmolar, partial hydatidiform mole, or complete hydatidiform mole.[1]

Determination of cell ploidy. Ploidy of molar tissue was examined in the early part of the study by cytogenetics[4] and later by distamycin/4′,6-diamidino-2-phenylindole staining of interphase nuclei.[4] Where ploidy had not been determined by these methods, flow cytometry of nuclei prepared from blocks of formalin-fixed, paraffin-embedded tissue was used.[5]

Genetic studies. Chromosomal and enzyme polymorphisms were examined as previously described.[2] For studies of restriction fragment length polymorphisms in molar and parental tissue, DNA was prepared by means of standard techniques and examined with Southern analysis. Hybridization was carried out with probes selected for Y-specific sequences and for unlinked sequences of DNA that showed a high degree of polymorphism (Table I).

Patient follow-up. Subsequent to the evacuation of the hydatidiform mole, the patients were monitored by estimation of human chorionic gonadotropin levels.[8] Thus it was possible to obtain details of follow-up.

Table I. Probes used to examine DNA in cases of molar pregnancy

Probe	*Chromosomal location*	*Allele*	*Polymorphisms*		*Heterozygosity (%)*	*Enzyme*
			Length (kg)	*Frequency*		
Single-copy probes[6]						
pHM6	4p11-q21	A1	4.8	0.50		*Eco*RI
		A2	4.6	0.50		
pCGα	6q12-q21 or	A1	10.0	0.36		*Eco*RI
	6p23-p21.1	A2	5.0	0.64		
		B1	4.0	0.65		*Hin*dIII
		B2	1.0	0.35		
pHC36	11p15.4	A1	6.5	0.66		*Taq*I
		A2	8.0	0.34		
p10-5	17p13.1	A1	5.35	0.29		*Hin*dIII
		A2	4.9	0.71		
Y chromosome–specific probes						
CY84[7]			5.8			*Eco*RI
48CB[7]			1.5			*Eco*RI
Locus-specific minisatellite probes[18]						
λMS1	1p35-p33		2.0-22*		98.0	*Hin*f1
λMS31	7pter-p22		3.5-13*		99.0	*Hin*f1
pλg3	7q36-qter		1.5-22*		97.0	*Hin*f1
λMS43	12q24.3-qter		3.5-16*		94.0	*Hin*f1

*Range.

Table II. Pathologic result and ploidy of hydatidiform moles

Pathologic type	*Ploidy*					*Total*
	Not known	*Haploid*	*Diploid*	*Triploid*	*Tetraploid*	
Complete	19	1	128	3*	—	151
Partial	4	—	1	42	2	49
Unclassified	—	—	2	—	—	2

*Two cases reclassifed as partial hydatidiform mole.

Results

Pathology. Of the 249 samples, 201 were classified as hydatidiform mole (151 as complete and 48 as partial); two hydatidiform moles could not be classified because the samples were inadequate (Table II). An additional case of a tetraploid conceptus[9] classified as partial hydatidiform mole on a review of the pathologic results was included in the study.

Ploidy. Ploidy of the molar tissue is shown in Table II. Twenty-six of the 49 partial hydatidiform moles were examined cytogenetically. Twenty-three were regular triploids and one was a tetraploid. The other two cases had the karyotypes 71,XXY,+C,+C[2] and 70,XXY,+mar,[4] respectively. None of the 40 complete hydatidiform moles examined cytogenetically deviated from a regular diploid karyotype.

By means of flow cytometry, three hydatidiform moles, classified pathologically as complete hydatidiform moles, were found to be triploid. Two of these cases were reclassified as partial hydatidiform moles on examination of further pathologic material from the referring hospital. One case of complete hydatidiform mole appeared to have only a haploid DNA complement on flow cytometry. In pathologic sections from the block used for flow cytometry, no evidence of tissue degradation was seen. The partial hydatidiform mole that was diploid by flow cytometry was reviewed but a pathologic diagnosis of partial hydatidiform mole was sustained.

Genetics

Partial hydatidiform moles. The parental origins and sex chromosome complements are shown in Table III. The triploid cases of partial hydatidiform mole were classified according to whether an origin by dispermy was proved (15 cases) or possible (15 cases). Where dispermy was possible, other mechanisms of origin that were not excluded are indicated in Table III. In all cases but one the additional chromosome set could be shown to be paternal in origin. In this case an origin by digyny could not be excluded. The tetraploid, with

Table III. Genetics of partial hydatidiform moles

Ploidy and origin			
Triploid		Tetraploid	
Total	44	Total	2
Origin not determined	14	Trispermy most likely	1
Dispermy proved	15	Equal maternal and paternal contributions	1
Dispermy possible	15		
or digyny	1	Diploid	
or patI	4	Total	1
or patII	4		
or patI/patII	6		
Sex chromosome complement			
Triploid		Tetraploid	
XXX	11	XXXX	1
XXY	12	Negative for Y-specific DNA sequences	1
XYY	1		
Positive for Y-specific DNA sequences	7	Diploid	
Negative for Y-specific DNA sequences	5	Negative for Y-specific DNA sequences	1
Not determined	8		

Table IV. Genetics of complete hydatidiform moles

Ploidy	
Total	149
Diploid	128
Triploid	1*
Haploid	1
Unknown	19
Genetic study	
Diploid and/or androgenetic	105
Heterozygous	13
Homozygous	39
Probably homozygous	29
Not informative	24

*Genetic studies showed one maternal and two paternal contributions to the genome.

Table V. Chemotherapy required after hyatidiform moles

Classification	*No. of cases*	*Resolved spontaneously*	*Treatment required*
Hydatidiform mole	202	175	27 (13%)
Partial hydatidiform mole	51	51	0
Complete hydatidiform mole	149	122	27 (18%)
Homozygous complete hydatidiform mole	39	30	9 (23%)
Heterozygous complete hydatidiform mole	13	10	3 (23%)

a 92,XXXX karyotype, had three paternal and one maternal chromosome sets, the most likely mechanism of origin being fertilization of an egg by three sperm.[9] In the other tetraploid case DNA analysis showed an equal genetic contribution from both parents (Fig. 1).

DNA analysis of the diploid partial hydatidiform mole showed both a maternal and paternal contribution to the molar sample. However, with all informative probes the paternal contribution appeared to be present in excess, suggesting that there might be two different populations of diploid cells present in the molar sample.

Complete hydatidiform moles. Genetic studies of the triploid complete hydatidiform mole showed one maternal and two paternal contributions, the situation usually associated with the pathology of partial hydatidiform mole. Of the 19 complete hydatidiform moles in which ploidy was not determined, genetic analysis showed that six cases were androgenetic in origin by excluding a maternal contribution to the genome. The 105 diploid and/or androgenetic complete hydatidiform moles have been divided into those where genetic studies were uninformative, those in which heterozygosity for one or more loci was demonstrated (heterozygous), those in which at least five markers for which the father was heterozygous were found to be homozygous in the molar tissue (homozygous), and those in which fewer than five markers for which the male parent was heterozygous were homozygous in the complete hydatidiform mole (probably homozygous). Of those where genetic origin was certain, 13 (25%) were heterozygous and 39 (75%) were homozygous (Table IV). Of the 13 heterozygous complete hydatidiform moles, eight were male, four were female, and sex was not determined in one.

Follow-up studies. Among the total 202 patients in this series, 27 (13%) subsequently required chemotherapy for a gestational trophoblastic tumor (Table V). In all 51 patients with partial hydatidiform mole, resolution of the molar pregnancy was spontaneous. Among the 149 patients with complete hydatidiform mole, 18% required chemotherapy, and among the subset of those patients in whom a definite genetic di-

agnosis was obtained, 23% required chemotherapy. No difference was found between patients with homozygous and heterozygous complete hydatidiform mole as far as the risk for development of gestational trophoblastic tumor is concerned.

Patients who required treatment were classified into risk groups that determine their initial chemotherapy.[10] The three patients with heterozygous complete hydatidiform mole who required treatment were initially in the low-risk category. Among the 24 treated patients with homozygous complete hydatidiform mole, 17 were assigned to the low-risk category and 7 to the medium-risk category. Two of the patients with heterozygous complete hydatidiform mole and six of the 14 patients with a genetic diagnosis of probable or definite homozygous complete hydatidiform mole originally treated in the low-risk category changed drug schedules because they failed to respond. All patients except one, who died of pulmonary embolism, responded to chemotherapy, and no sequelae have been reported.

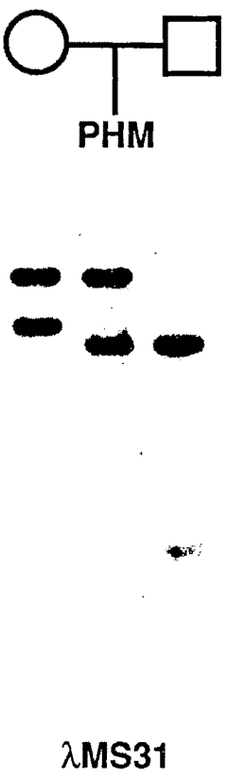


Fig. 1. Restriction fragment length polymorphisms in tetraploid partial hydatidiform mole *(PHM)* showing an equal contribution from each parent.

Comment

In this study we accepted samples whenever a diagnosis of hydatidiform mole had been suspected on clinical grounds, but in 48 cases the diagnosis was not sustained pathologically. Assignment to the partial or complete mole category was difficult with small samples, particularly in the case of partial hydatidiform moles, where some sections of the tissue may appear normal and others may have the features of complete hydatidiform mole. Two cases remained unclassified.

The only accurate way of determining the precise karyotype of molar tissue is by cytogenetics, which necessitates fresh tissue and a successful culture to obtain metaphase spreads. However, an assessment of ploidy can be made by other techniques. The introduction of flow cytometry has been a useful advance because the method can be applied to cells recovered from formalin-fixed, paraffin-embedded material. Most molar samples are contaminated with some maternal tissue, whcih appears as a diploid peak on flow cytometry.[5] In triploid partial hydatidiform moles this provides a control diploid population. In the case of diploid complete hydatidiform moles a smaller tetraploid peak, which represents dividing molar cells, helps confirm that some of the diploid tissue is molar. In tetraploid hydatidiform moles the maternal diploid population is smaller, and an octoploid population of dividing molar cells may be identified.

Genetic polymorphisms can also be used to determine ploidy. For example, if three alleles are shown to be present at a single locus, it is diagnostic of trisomy (Fig. 2, *a*). If only two alleles are identified but one is present at a higher dose than the other (Fig. 2, *b*), this can again be taken as evidence of the trisomic state. The identification of trisomy with several markers would suggest triploidy.

Cytogenetics, enzyme polymorphisms, and restriction fragment length polymorphisms (Fig. 2) have demonstrated that partial hydatidiform moles usually have two paternal contributions and one maternal contribution to the genome. Genetic fingerprinting can be used to identify parental DNA, but the relative contributions of each parent may be difficult to determine. Examination of locus-specific minisatellite polymorphisms that are unlikely to be shared by parents provides a more appropriate means of determining the parental contributions. Since these probes identify telomeric sequences[11] that are likely to have undergone recombination during meiosis, the precise origin of partial hydatidiform moles cannot be determined with these probes. Cytogenetics, where polymorphisms are usually centromeric, was the only technique with which we were able to prove dispermy as the mode of origin. Use of molecular genetic probes for centromeric regions would enable the origin of partial hydatidiform moles to be determined by DNA analysis.

If all triploid partial hydatidiform moles arise by dispermy, the expected ratio of XXX/XXY/XYY

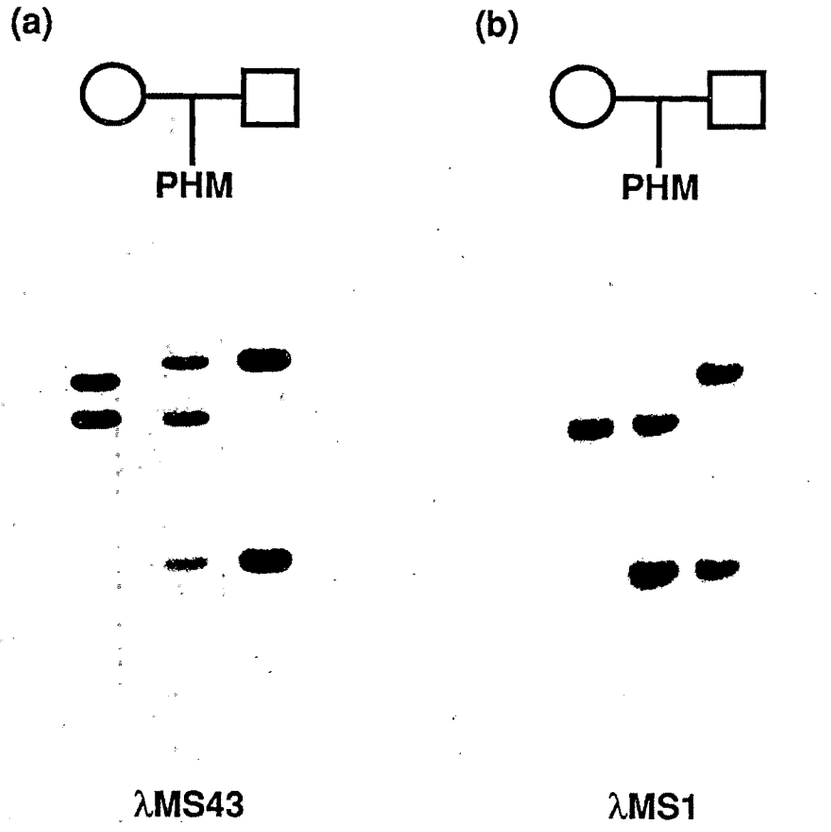


Fig. 2. a, Restriction fragment length polymorphisms in partial hydatidiform mole *(PHM)* showing two paternal and one maternal allele. **b,** Restriction fragment length polymorphisms in same partial hydatidiform mole showing greater paternal than maternal contribution.

would be 1:2:1, whereas in this series the ratio was 11:12:1. The deficiency of XYY has been observed among other series of triploid conceptions[12] and is assumed to be due to poor survival. The observed 1:1 ratio of XXX/XXY in this series might be explained if, where dispermy was not proved, triploidy was due to an error at patII producing XXX partial hydatidiform moles. However, a 1:1 ratio of XXX/XXY, rather than the 1:2 ratio expected, also was found among partial hydatidiform moles proved to arise by dispermy. It is possible that XXY partial hydatidiform moles are more subject to early loss by spontaneous abortion than are XXX partial hydatidiform moles.

The few previously described tetraploid conceptions with the pathologic features of partial hydatidiform mole had three paternal chromosome sets; therefore, like triploid partial hydatidiform moles, they have more paternal than maternal sets of chromosomes.[13] One tetraploid partial hydatidiform mole in this series apparently had an equal genomic contribution from each parent with the probes used (Fig. 1).

The case in this series in which the morphologic result was clearly partial hydatidiform mole and flow cytometry suggested diploidy appeared on molecular genetic studies to have a greater paternal contribution to the genome, compatible with triploid partial hydatidiform mole. These results suggest that two populations of cells might be present and that the conceptus was possibly hydatidiform mole with a nonmolar twin. In this case a cytogenetic study could have resolved the problem if cells from both populations had been established in tissue culture. Two sets of twins were identified unequivocally in our data.

In complete hydatidiform moles different polymorphic systems have been used to demonstrate androgenesis. The disadvantage of cytogenetics is that a result depends on successful tissue culture of molar cells. The best method of demonstrating a maternal exclusion is by use of locus-specific minisatellite probes (Figs. 3 and 4). The enzyme systems and the single-copy DNA sequences are not very polymorphic, and it may sometimes be difficult to distinguish between maternal contamination and a maternal contribution to the genome of the hydatidiform mole. With the polymorphisms identified by locus-specific minisatellite probes, maternal contamination can usually be identified as two bands

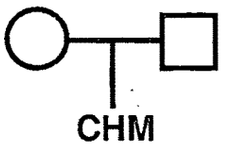




Fig. 3. Restriction fragment length polymorphisms in complete hydatidiform mole *(CHM)* showing only paternal contributions. It is homozygous for one of two paternal alleles.

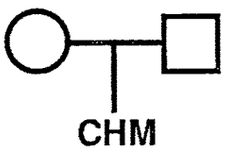




Fig. 4. Restriction fragment length polymorphisms in a complete hydatidiform mole *(CHM)* showing only paternal contributions. Genomes of male parent and complete hydatidiform mole are heterozygous.

weaker than those in the molar tissue, whereas a maternal contribution would generally give a single band.[7]

The Y chromosome or Y chromosome–specific sequences are useful for the identification of 46,XY heterozygous complete hydatidiform moles. To exclude the possibility of heterozygosity in 46,XX complete hydatidiform moles, it is also necessary to examine at least four autosomal loci for which the male parent is heterozygous. The probability that a complete hydatidiform mole with five homozygous markers has arisen by dispermy is then 1 in 32. Although DNA fingerprinting may demonstrate androgenesis, because of the complexity of the band patterns, it is not usually possible to distinguish with certainty between a homozygous and heterozygous complete hydatidiform mole with this technique. However, demonstration of two different paternal alleles with a locus-specific probe is proof of heterozygosity (Fig. 4).

The frequency of heterozygous complete hydatidiform moles in this series was 16%. Since there may be undetected heterozygous complete hydatidiform moles among the 46,XX cases tested for fewer than five informative markers, 16% is the minimum frequency of dispermic complete hydatidiform moles. A similar frequency of heterozygous complete hydatidiform moles has been observed in Japan.[14] The sex ratio 2:1 in the series XY/XX would be expected on the assumption that a YY conceptus is nonviable.

The patients in this series who required treatment for a gestational trophoblastic tumor were ascertained on the basis of human chorionic gonadotropin studies.[8] Since the diagnosis of gestational trophoblastic tumor was not based on pathologic results, the distinction between benign invasive mole and malignant choriocarcinoma could not be made. The molar pregnancy resolved spontaneously in all 51 patients with partial hydatidiform mole. This does not mean that there is no chance of a gestational trophoblastic tumor after partial hydatidiform mole, but it does confirm that the risk is low. Recently the incidence of therapeutic intervention after partial hydatidiform mole was estimated to be of the order of 1 in 200.[15] Unequivocable proof of the development of choriocarcinoma following pregnancy with partial hydatidiform mole is still lacking. It must

always be borne in mind that, although a gestational trophoblastic tumor is usually related to the immediately antecedent pregnancy, tumors can arise from earlier pregnancies (Fisher, RA. Unpublished data).

In this series there was no difference in the frequency with which patients with homozygous or heterozygous complete hydatidiform mole required treatment. This point is of interest because evidence from Japan suggests that patients with heterozygous complete hydatidiform mole have a much higher risk of development of gestational trophoblastic tumor than those with homozygous complete hydatidiform mole.[14] Although in our series we could not determine whether the gestational trophoblastic tumor was invasive mole or choriocarcinoma, we have shown by genetic studies of trophoblastic tumors that choriocarcinoma can develop from either a homozygous or heterozygous complete hydatidiform mole[16] (Fisher RA. Unpublished data).

As far as the response to treatment is concerned, we have not observed that the homozygosity or heterozygosity of a complete hydatidiform mole is a prognostic factor, since some of the patients in both categories required chemotherapy with more aggressive regimens than methotrexate and folinic acid.

Genetic studies in experimental animals have shown that the maternal and paternal genomes have different functions during the development of the embryo, because of differential gonadal imprinting.[17] In nuclear transplantation experiments in mice, only eggs with one male and one female pronucleus develop to term, indicating that both parental genomes are necessary for normal development.[17] In abnormal embryos with two male pronuclei, trophoblast developed relatively well whereas in those with two female pronuclei embryonic development was favored.[18]

This situation has a parallel in hydatidiform moles. In both complete and partial hydatidiform moles the presence of two paternal chromosome sets is associated with hyperplasia of the trophoblast; a triploid conceptus with one paternal and two maternal sets is not usually associated with a pathologic diagnosis of partial hydatidiform mole.[12] The diploid androgenetic complete hydatidiform mole shows a greater degree of trophoblastic hyperplasia, and an embryo does not develop. As in mice, androgenesis favors trophoblastic proliferation. In triploid partial hydatidiform mole the presence of a maternal contribution to the genome is associated with initiation of the development of an embryo, although it usually fails to develop properly.

The contribution of the paternal genome in relation to trophoblastic hyperplasia is also evident in the rare types of triploid and tetraploid hydatidiform moles.[13] Tetraploids without a maternal genome have the pathologic features of complete hydatidiform moles,[13] whereas if a maternal genome is present the pathologic results usually conform to partial hydatidiform mole. In cases of tetraploidy with the pathologic features of partial hydatidiform mole that apparently have equal genomic contributions from each parent, only some chromosomes have been examined. Thus it cannot be excluded that there are genetic regions, which are concerned with trophoblastic proliferation, where paternal alleles are present in excess. A similar situation occurs in patients with genetic disorders who, although having a normal diploid karyotype, have regions of the genome that are derived from only one parent, a condition termed *uniparental disomy*.[19] This could possibly also account for those rare cases where an apparently normal diploid conceptus is associated with molar pathologic results.[13]

The effect of genomic imprinting also applies to gestational trophoblastic tumor in that an androgenetic complete hydatidiform mole is associated with a greater risk of the development of gestational trophoblastic tumor than a normal conceptus, whereas in partial hydatidiform mole, which has one maternal and two paternal sets of chromosomes, the risk is intermediate. Thus both the pathologic features of hydatidiform mole and the risk of gestational trophoblastic tumor are related to the ratio of maternal to paternal genomes in the conceptus.[20]

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Additional references are available from the authors on request.

The effect of sterile water blocks on low back labor pain

Birgitta Trolle, MD, Margrethe Møller, MD, Hanne Kronborg, and Søren Thomsen, MD
Aalborg, Denmark

To evaluate the analgesic effect of intradermal sterile water blocks, 272 women in labor complaining of severe low back pain were randomly assigned to treatment with either sterile water or saline solution blocks. Pain intensity was assessed on a visual analog scale, before the blocks were given and again 1 and 2 hours later. The groups were equal with regard to age, parity, fetal size, progression of labor, and initial pain scoring. Pain scoring 1 and 2 hours after the blocks were given showed a significantly higher degree of analgesia in the sterile water group. No adverse effects were noted, and patient acceptability was high. (AM J OBSTET GYNECOL 1991;164:1277-81.)

Key words: Obstetric analgesia, low back labor pain, sterile water blocks

About one third of women in labor have severe, continuous, low back pain, and an additional number feel the pain of contractions primarily in the back.[1] To reduce this pain, a number of analgesic methods have been tried with varying success, i.e., transcutaneous nerve stimulation,[2,3] epicutaneous application of a local anesthetic agent,[4,5] and intracutaneous or subcutaneous infiltration of the skin with procaine hydrochloride (Novocain).[6,7]

Intradermal injection of small amounts of sterile water, called sterile water blocks, was reportedly effective against ureteral colic,[8] and in this article we present the result of a double-blind, randomized trial comparing the analgesic effects of intradermal sterile water blocks with those of isotonic saline solution blocks on low back labor pain.

Material and methods

Women in active labor complaining of severe low back pain were asked to participate and given detailed

From the Department of Obstetrics and Gynecology, Aalborg Hospital.
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Reprint requests: Birgitta Trolle, MD, Hamar Sjukehus, N-2300 Hamar, Norway.
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Table I. Maternal characteristics for sterile water and groups

	Sterile water (n = 141)	*Saline solution (n = 131)*
Primiparous women (%)	70.2 75.6	
Gestational length (wk)	39.9 ± 1.6	39.7 ± 2.1
Maternal age (yr)	26.2 ± 5.1	25.9 ± 3.9
Cervical dilatation (cm)	4.73 ± 1.91	4.68 ± 1.91
Ruptured membranes (%)	59.6	52.7

Numbers are mean ± SD or percent. There are no significant differences.

Table II. Analgesic effects of sterile water and saline solution intradermal blocks on low back pain

	Sterile water	*Saline solution*	*p Value*
Immediately before blocks	83 (29-100) (*n* = 141)	81 (31-100) (*n* = 131)	0.23
After blocks			
1 hr	29.5 (0-100) (*n* = 132)	76 (0-100) (*n* = 121)	1×10^{-7}
2 hr	53.5 (0-100) (*n* = 100)	82 (1-100) (*n* = 99)	4.5×10^{-6}

Pain scoring (mean and range) and result of significance test before blocks and 1 and 2 hours later. As some women were delivered, groups diminished.

verbal and written information about the study. Those who accepted were asked to assess pain severity on a visual analog scale, a horizontal line 10 cm long without any markings, except the number *0* and the words *no pain* at the left end and the number *10* and *unbearable pain* at the right. This has proved to be a reliable method of measuring pain intensity.[9] The midwife in charge performed a vaginal examination, noting cervical dilatation. Sterile water and isotonic saline solution were provided in identical 1 ml ampules that had been randomly mixed and numbered. The midwife used the ampule next in number, giving the blocks by injecting about 0.1 ml intradermally at four different spots in the low back area, approximately corresponding to the borders of the sacrum.

The participants were asked whether they noted any analgesic effect from the blocks and, if so, the length of time before the effect began. Pain scoring and vaginal examination were repeated 1 and 2 hours after the injections if the woman had not yet been delivered. Apart from the blocks, the women were treated according to the usual principles of the department; they were offered nitrous oxide or pethidine if pain was severe. Any use of analgesics during the 3-hour period before block placement and the two subsequent hours was registered. Paracervical block and epidural anesthesia were not available. The water blocks could be given only once to each patient and only to those patients participating in the study.

Table III. Number of instrument-assisted deliveries specified in cesarean sections and vacuum extractions in sterile water and saline solution groups

	Sterile water (n = 141)	*Saline solution (n = 131)*	*p Value*
Cesarean section	6	15	<0.05
Vacuum extraction	22	13	NS
Total No. of instrument-assisted deliveries	28	28	NS

After delivery the patients were asked if they would request the same type of analgesia at subsequent delivery.

Clinical data of patients and infants and all complications during labor and delivery were registered, and a χ^2 test was used for comparison of frequencies. The scores on the visual analog scales were recorded as the distance in millimeters from point 0, and for each of the hourly intervals the groups were compared by a Mann-Whitney test. A p value of 0.05 was chosen as a significant level.

The study was approved by the local ethical committee.

Results

Two hundred seventy-two women received the blocks and completed the answer forms.

Sterile water blocks were given to 141 women and saline solution was given to 131 women. The groups were equal with regard to number of primiparous women, maternal age, and length of gestation and also with regard to mean cervical dilatation and number of patients who had ruptured membranes when the blocks were given (Table I).

Before they entered the study, 9.9% of the women in the sterile water group had already received pethidine in a standard dose of 75 mg intramuscularly, and 3.5% had received nitrous oxide. Corresponding figures for the saline solution group were 6.9% and 3.1%, respectively. In the sterile water group 126 (89.4%) of the women noted an analgesic effect of the blocks, compared with 59 (45%) in the saline solution group ($p < 0.0005$). The median number of minutes before the effect was apparent was 2 (range, 1 to 30) for both

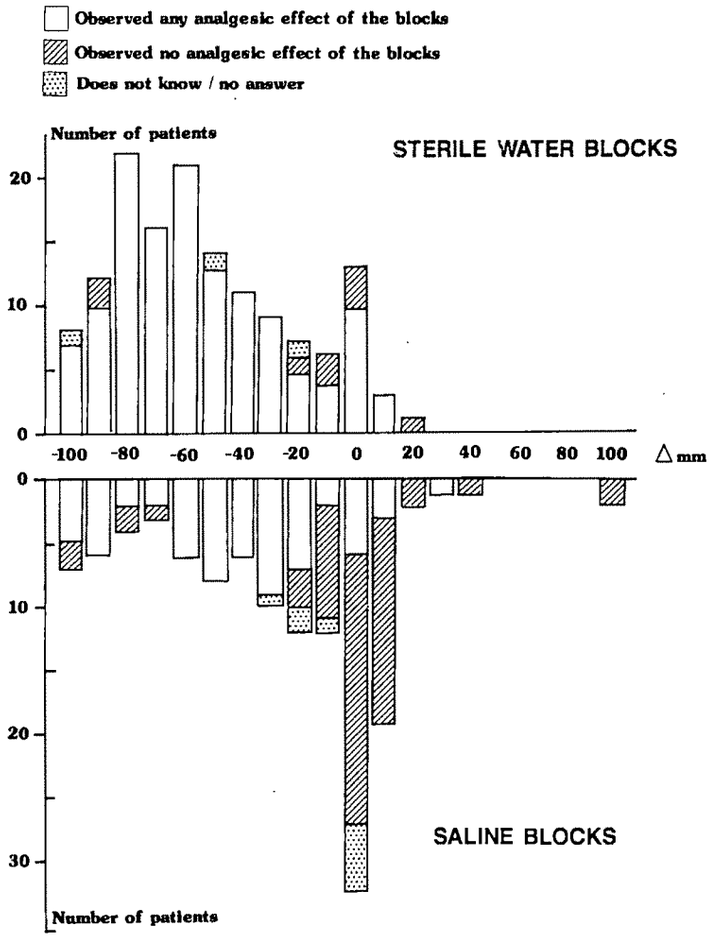


Fig. 1. Change in pain scoring after sterile water or saline solution blocks for low back labor pain, in relation to patients' answers to questions of any observed analgesia. Change in millimeters (Δ *mm*) is calculated as 1-hour scoring on the visual analog scale minus scoring before treatment.

groups. Initial pain scoring did not show any significant difference between the groups, but 1 and 2 hours after the blocks were given, there were highly significant differences (Table II).

During the 2-hour observation interval after the blocks were given, 6.4% of the patients in the sterile water group needed pethidine, and 12.8% received nitrous oxide, compared with 8.4% and 16%, respectively, in the saline solution group. These frequencies do not differ significantly.

Fig. 1 is a graphic illustration of the results. It shows the difference in analgesic effect between the groups but also demonstrates that even if no analgesia was noted, low back pain was at least constant and not increasing in spite of progressing labor. Except for the initial burning sensation, lasting for about 30 seconds, no local side effects were noted.

Progression of labor, expressed as time from application of the blocks until full cervical dilatation, was equal in the two groups (median, 2 hours; range, 0 to 11 hours). The second stage of labor had a median duration of 0.5 hours (range 0 to 2.25 hours) in both groups.

The rate of dystocia necessitating oxytocin stimulation was 39.7% in the saline solution group and 32.6% in the sterile water group. Although the rate of instrument-assisted delivery was similar in the two groups, the rate of cesarean section was significantly higher in the saline solution group (Table III). This was caused predominantly by a higher incidence of cephalopelvic disproportion and malposition of the occiput (Table IV). At vaginal delivery the rate of malposition of the occiput was 9.5% in the saline solution group and 5.2% in the sterile water group. The difference is not significant. There was no difference in average blood loss. Infant birth weight and Apgar scores also were equal.

A significantly greater proportion of the women in

Table IV. Indication for operative delivery in sterile water and saline solution groups

	Sterile water	*Saline solution*
Threat of asphyxia	11 (0)	5 (1)
Cephalopelvic disproportion	5 (5)	12 (10)
Lack of progression	4 (1)	0
Malposition of the occiput	1 (1)	10 (9)
Exhausted mother	11 (1)	8 (0)
Other	1 (1)	1 (1)

Numbers in parentheses indicate cesarean section. Some operations were performed for more than one indication. The total number of operative deliveries was 28 in each group.

Table V. Answer to question, "Will you request the same kind of analgesia if you are going to deliver again?" in sterile water and saline solution groups

	Sterile water (n = 141)	*Saline solution (n = 131)*
Yes	96	66
No	18	35
Undecided–no answer	27	30

$p < 0.005$, χ^2 test.

the sterile water group than in the saline solution group would request the same type of analgesia if they again had to undergo delivery ($p < 0.005$) (Table V).

Comment

Danish women do not expect childbirth to be painless. Epidural analgesia is available as a fulltime service in only a few centers. The use of paracervical block was largely abandoned after reports of fetal bradycardia, and pethidine is used mostly for women complaining of severe pain in early labor. Nitrous oxide is the most widely used analgesic, and pudendal block is offered to all women. Psychoprophylaxis is regarded as important, and most primigravid women attend free antenatal classes. Many primigravid, and most multigravid, women are delivered without any request for analgesia, and we find it reasonable to assume that the women who chose to participate in this study felt severe low back labor pain.

Pain impulses from the posterior part of the cervix and uterus are transmitted to the spinal cord segments T11-L1, and cutaneous branches from these segments supply the skin over the low back area.[10] This is probably the anatomic background of how pain originating in the cervix can be localized to the back. Low back pain occurs often in labor, especially in connection with an occipitoposterior presentation, where stretching of the posterior part of the lower uterine segment and cervix may be predominant. The hypotonic sterile water injected intradermally causes local irritation and thereby a strong sensory stimulation of the surrounding skin area for about 30 seconds. The analgesia induced by this stimulation may originate in midbrain centers,[11, 12] or be caused by "gate control" at the spinal level.[13, 14] Hyperstimulation of a skin area can affect perception of visceral pain, and it appears that the mechanism of referred pain can be reversed to produce referred analgesia.

Intradermal injection of isotonic saline solution is less painful than sterile water but not painless, and saline solution blocks could theoretically induce some degree of analgesia. To make the study double-blind some kind of injection in the low back area had to be used in the treatment of the control group. Comparing the blocks with any of the established methods of analgesia was abandoned for the same reason. These circumstances make it impossible to state the number of patients having only a placebo effect of the blocks.

A great number of women joined the project. A study of this size has a power of almost 90% in detecting a difference in proportions between the two groups of at least 15%.

The highly significant difference in pain intensity between the groups after the blocks were given is not reflected in the use of pethidine, which was similar in the two groups. The number of women receiving pethidine is small—smaller than the total number of women who did not have any analgesic effect of the blocks. It is not possible to make any conclusions.

We found it important to certify that the analgesic effect of the blocks was not connected with an impairment of labor, as this would only postpone pain. No such effect was noted. The incidence of dystocia and oxytocin infusion was high in both groups, but this was to be expected because only women with low back pain entered the study and low back pain commonly occurs with malrotation of the fetus.

There was no difference between the groups with regard to the rate of instrument-assisted delivery. However, the rate of cesarean section in the saline solution group was similar to the usual rate of cesarean section in our department (3000 deliveries per year), whereas the rate of cesarean section in the sterile water group was significantly lower (4.2% vs 11.4%). We have no definite explanation for this. The most frequent indications for cesarean section in the saline solution group were malposition of the occiput and cephalopelvic disproportion. In the Danish population the latter condition is usually relative, reflecting a fetal malrotation. It is not possible to determine if there was a primary difference between the study groups regarding fetal position or a higher rate of correction of fetal position in the sterile water group. Generally, the relation be-

tween labor pain and fetal position and the effect of analgesia on pelvic floor tone, cervical tension, contraction pattern, and fetal rotation are interesting and an evident subject for further studies.

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Electronic fetal heart monitoring, auscultation, and neonatal outcome

Patricia H. Ellison, MD,[a] Mark Foster, MA,[a] Margaret Sheridan-Pereira, MD,[b] and Dermot MacDonald, MD[c]

Denver, Colorado, and Dublin, Ireland

In a large randomized, controlled study of fetal heart rate monitoring with either continuous electronic fetal heart monitoring or auscultation at specified intervals, only one pattern of deviation in the fetal heart rate correlated significantly with neonatal neurologic examinations at 0 to 48 hours and 72 hours to 1 week: late decelerations in stage 1 and in stage 2. Other variables from labor and delivery, specifically, duration of labor after hospital admission, failure of labor to progress, number of fetal scalp pH values, and presence of meconium were important predictors of neonatal outcome in the regression analyses. The fetal heart rate deviations did contribute significantly to the percent variance accounted for in the regression analyses with neonatal outcomes of Apgar scores at 1 and 5 minutes and serial neonatal neurologic examinations. (Am J Obstet Gynecol 1991;164:1281-9.)

Key words: Continuous electronic fetal heart monitoring, late decelerations, Apgar scores at 1 and 5 minutes, neonatal neurologic examination

Electronic fetal heart monitoring, when introduced in the 1960s and refined in subsequent years, brought hope that there could be a way to detect, in utero, an already compromised fetus, as well as a fetus in the process of deterioration, particularly during labor and delivery. In certain situations this technology has been fairly reliable, e.g., in the recognition of a dying fetus who previously might have been stillborn. It has been less reliable in the detection of fetuses with hypoxia-ischemia of the central nervous system and secondarily decreased regulation of heart rate.

In the majority of studies the relation was examined between fetal heart rate and the Apgar score, usually at 1 and 5 minutes. Very few studies have examined

From the Department of Psychology, University of Denver,[a] and Coombs Hospital[b] and National Maternity Hospital,[c] Dublin.
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Reprint requests: Patricia H. Ellison, MD, Research Professor, Department of Psychology, University of Denver, Denver, CO 80208.
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Table IA. Perinatal variables for four subsamples in percentages

	Auscultation (n = 2362)	*Electronic fetal heart monitoring (n = 2362)*	*Auscultation plus neurologic examination (n = 135)*	*Electronic fetal heart monitoring plus neurologic examination (n = 135)*
Condition of mother				
Eclampsia	1.6	1.9	6.6	6.8
Diabetes	1.0	1.2	3.6	6.8
Failure of labor to progress	5.8	9.0	11.6	15.5
Treatment				
Induction of labor	16.8	11.7	26.8	18.9
Use of pethidine	46.6	53.8	46.4	54.7
Use of epidural anesthesia	3.3	3.8	3.6	2.7
Use of oxytocin	22.2	28.3	23.9	41.9
Use of forceps	5.8	13.8	11.6	25.0
Use of cesarean section	1.5	2.4	7.2	1.4
Condition of fetus				
Postterm	5.5	4.3	5.1	2.0
Breech	1.8	1.0	8.0	2.7
Sampling of scalp pH	0.9	6.9	2.2	15.6
Abnormal heart rate	1.1	5.9	5.1	10.8
Presence of meconium	11.3	10.0	15.2	10.8
Condition of neonate				
Stillborn	0.4	0.0	0.0	0.0
Neonatal death	0.9	1.8	0.7	0.0
Neonatal convulsions	0.3	0.2	3.6	4.1

the relation between fetal heart monitoring and neonatal neurologic examination obtained in a standardized manner.[1-9] However, the general course of most compromised newborns is improvement from the minute of birth through the hours or days until such examinations are performed, especially with expert resuscitation and neonatal care.

In this study we use the data from the Dublin randomized, controlled trial[5] to examine the relationships between a number of maternal and labor-and-delivery variables, including fetal heart rate and different fetal heart patterns, to several neonatal outcomes. The questions posed were: (1) Which specific fetal heart patterns are related to neonatal outcome as reflected in the Apgar scores at 1 and 5 minutes, the presence of convulsions, and the measures based on neurologic examination at 0 to 48 and 72 hours to 1 week? (2) Which other labor-and-delivery variables are related to these neurologic outcomes? (3) How much does each of these variables contribute to the prediction of outcome? (4) On which of these indicators do obstetricians base interventions, such as use of forceps or cesarean section?

All newborns in this study were examined by a physician before discharge. Approximately 1000 were examined neurologically by a research physician using a standardized method. These newborns constituted all those admitted to the special care baby unit, all those with any neurologic abnormality on routine examination, and all those of special concern to the nursing staff. Every effort was made to examine in a standardized manner all newborns at risk for or suspected to have neurologic abnormality. The subsamples available for various analyses differed in size. However, all subsamples were not only larger than those in most such studies but sufficiently large to consider that the results were likely to represent the population well.

Methods

The data in this study were those from the Dublin trial, a randomized, controlled study that compared the effectiveness of electronic fetal heart monitoring and auscultation in assessing the health of the fetus during labor and delivery.[5] There were 12,964 women involved in the study at the National Maternity Hospital in Dublin between March 1981 and April 1983. Data for 10,089 deliveries were sent to the University of Denver for analyses. Women who had heavily meconium-stained or markedly decreased amniotic fluid or who had fetuses with abnormal heart rates on admission were not enrolled. The women were randomly assigned to assessment by continuous electronic fetal heart monitoring or to intermittent monitoring by auscultation. Fetal scalp sampling was available to both sections.

Four subsamples were used in the results described in this article. The auscultation-only group was a random sample of all deliveries for which data were sent and in which only auscultation of the fetal heart was performed (n = 2362). The electronic fetal heart monitoring group, which was equal in number to the auscultation-only group, included all deliveries assigned to electronic fetal heart monitoring for which data were available for both stages 1 and 2 (n = 2362). The auscultation-plus-neurologic group, a random sample equal to that of those babies who had electronic fetal heart monitoring in stages 1 and 2 and a

Table IB. Mean Apgar scores and duration of labor

	Auscultation (n = 2362)	*Electronic fetal heart monitoring (n = 2362)*	*Auscultation plus neurologic examination (n = 135)*	*Electronic fetal heart monitoring plus neurologic examination (n = 135)*
1 min	8.8 ± 1.0	8.7 ± 1.0	7.6 ± 2.4	7.6 ± 2.1
5 min	9.1 ± 0.6	9.1 ± 0.6	8.7 ± 1.1	8.7 ± 1.2
Duration of labor (after entering hospital)	3.5 ± 2.6	4.2 ± 2.6	3.7 ± 2.8	4.9 ± 3.1

neurologic evaluation at 0 to 48 hours and 72 hours to 1 week, was from those deliveries that were monitored only by auscultation and in which the babies underwent a neurologic examination by the research physician at both 0 to 48 hours and 72 hours to 1 week (n = 135). The electronic fetal heart monitoring–plus-neurologic group, equal in number to the auscultation-plus-neurologic group, included those neonates whose mothers were assigned to electronic fetal heart monitoring, had such data during stages 1 and 2, and had both neurologic examinations by the research physician (n = 135). We wanted to compare samples of equal size and used random sampling to form the subsamples. In the regression analyses the sample sizes of each of these groups decreased slightly because of missing data.

A large number of maternal, fetal, and labor-and-delivery variables were recorded, including maternal age, parity, gravidity, gestational age of fetus, use of oxytocin or pethidine, length of time from admission to delivery, failure of labor to progress, delivery by cesarean section or forceps, and Apgar scores at 1 and 5 minutes.

The fetal heart tracings were interpreted by an obstetrician who had no knowledge about the mother, the other information about the labor and delivery, or the condition of the baby. The categories were marked bradycardia, late deceleration pattern, severe variable deceleration pattern, marked tachycardia, moderate tachycardia with reduced variability, moderate bradycardia with reduced variability, minimal variability, moderate variable decelerations, moderate tachycardia with normal variability, mild variable deceleration pattern, early deceleration pattern, reduced variability, moderate bradycardia with normal variability, acceleration pattern, normal baseline and variability, and unclassifiable (poor quality, labor too short, monitor disconnected, and not known—trace missing).

All newborns were examined physically and neurologically before discharge by a physician. Those newborns for whom there was concern about neurologic dysfunction by physician or nurse for any reason or who were admitted to the neonatal intensive care unit were examined by one physician using a comprehensive neonatal neurologic examination. This included individually scored items from the Dubowitz,[10] Prechtl,[11] and Brazelton[12] methods of evaluation, as well as items for gestational age scoring. The babies were examined at 0 to 48 hours, at 72 hours to 1 week, and at weekly intervals until discharge. The overall examination result was scored as normal, questionably abnormal, or abnormal. Data also were recorded about neonatal death, congenital abnormalities, and neonatal convulsions. The neurologic data were subsequently analyzed extensively and used to construct a neonatal neurologic assessment instrument with a total score and seven subscores.[13]

Frequencies were reviewed for all variables, as well as distributions and skews. Pearson product moment correlations and point biserial correlations (for dichotomous variables) were obtained and reviewed among all variables for each sample. Multiple regression analyses were obtained for different outcomes: Apgar scores at 1 and 5 minutes and neurologic examination at 0 to 48 hours and 72 hours to 1 week. The variables entered in the regression analyses for the two auscultation groups were confined to duration of labor, presence or absence of meconium, failure of labor to progress, use of oxytocin, number of fetal scalp samples, and any fetal heart rate abnormality. The regression analyses for the two electronic fetal heart monitoring groups were confined to the first five variables as noted above plus the ominous (marked bradycardia, late deceleration pattern, and severe variable deceleration pattern) and suspicious (marked tachycardia, moderate tachycardia with reduced variability, moderate bradycardia with reduced variability, minimal variability, and moderate variable decelerations) fetal heart patterns entered separately for stages 1 and 2. The ominous and suspicious rankings were determined by the obstetric classifications. The decision to select variables for entry in the regression analyses were based on review of the correlation matrices. Finally, regression analyses were obtained to determine those labor variables that accounted for obstetric intervention—use of forceps or cesarean section. The variables entered in the regression analyses were restricted to those described above for the four samples.

Results

The frequencies of key variables including eclampsia, diabetes, and breech position for the several subsam-

Table II. Frequencies of specific fetal heart monitoring patterns in percent

	Stage 1		*Stage 2*	
	Electronic fetal heart monitoring	*Electronic fetal heart monitoring plus neurologic examination*	*Electronic fetal heart monitoring*	*Electronic fetal heart monitoring plus neurologic examination*
Marked bradycardia	1.5	0.7	14.0	12.8
Late decelerations	1.8	3.4	4.7	10.1
Severe variable decelerations	1.4	3.4	2.0	2.0
Marked tachycardia	0.4	1.4	0	0
Moderate tachycardia with reduced variability	0.2	2.0	0	0
Moderate bradycardia with reduced variability	0	0	0	0
Minimal variability	0.4	0.7	0	0
Moderate variable decelerations	0.9	0.7	2.1	2.7
Moderate tachycardia with normal variability	3.2	5.4	1.1	2.7
Mild variable decelerations	15.3	20.3	12.6	14.9
Early deceleration pattern	11.2	15.5	39.5	33.1
Reduced variability	3.0	3.4	0.4	0.7
Moderate bradycardia with normal variability	10.7	6.1	6.6	5.4
Acceleration pattern	4.5	4.1	0.5	0.7
Normal baseline and variability	45.6	33.1	16.4	13.5
Unclassifiable	0	0	0	1.4

ples are shown in Table IA. Intervention by use of forceps is low, with the exception of the electronic fetal heart monitoring–plus–neurologic examination subsample. The rate of cesarean section is also low, particularly in comparison with that of American samples. The means and standard deviations for Apgar scores at 1 and 5 minutes and for the duration of labor after hospital entry are shown in Table IB. The 1- and 5-minute Apgar scores were somewhat lower and the standard deviations larger for the two subsamples with neurologic examination. The duration of labor was longest for the electronic fetal heart monitoring–plus–neurologic examination subsample.

The frequencies of fetal heart monitoring patterns are shown in Table II for the subset with electronic monitoring. The frequencies for the patterns that have been characterized as ominous are low, with the exception of marked bradycardia in stage 2 labor. The frequency of patterns that have been characterized as "nonreassuring" is high during stage 2 labor, especially for early deceleration. The frequency of normal baseline and variability decreases to only a small percentage of fetuses by stage 2. Thus the majority of patterns were described as "other than normal."

In Table IIIA the few correlations with significance are shown between those fetal heart patterns and outcomes. The most significant correlations were those between late decelerations (stage 1) and the development of convulsions and two neurologic examinations. There also were statistically significant correlations between late decelerations (stage 2) and the development of convulsions and two neonatal neurologic evaluations, but they were smaller. The correlations between presence of meconium, duration of labor, failure to progress, use of oxytocin, and number of scalp pH values and the several outcomes are shown in Table IIIB. The only significant correlation with the second neurologic evaluation is presence of meconium.

In Table IV the results are shown for the regression analyses with Apgar score at 1 minute as the dependent variable. In all the subsamples the percent variance accounted for ranged from 3.2 to 24.3. The heart rate abnormalities were significant in adding to the prediction in the auscultation, electronic fetal heart monitoring, and electronic fetal heart monitoring–plus–neurologic examination groups. However, larger contributions were made when specific patterns were used as in the electronic fetal heart monitoring and electronic fetal heart monitoring–plus–neurologic examination groups. The specific patterns combined to make the largest contribution in the electronic fetal heart monitoring–plus–neurologic examination group; those patterns were minimal variability (stage 1), marked bradycardia (stage 2), and late decelerations (stage 2). In the electronic fetal heart monitoring group, late decelerations (stage 2) was the most powerful predictor variable; in the electronic fetal heart monitoring–plus–neurologic examination group, marked bradycardia (stage 2) was the lead or most powerful predictive variable.

In Table V the results are shown for the regression analyses with Apgar score at 5 minutes as the dependent variable. In the auscultation and electronic fetal heart monitoring groups extremely small amounts of the variance were accounted for. In the subsamples evaluated neurologically, a larger percent of the vari-

Table IIIA. Correlations of specific fetal heart patterns to outcomes

	Electronic fetal heart monitoring		*Electronic fetal heart monitoring plus neurologic examination*		
				Neurologic examinations	
	Apgar score at 1 min (n = 2362)	*Apgar score at 5 min (n = 2362)*	*Convulsions (n = 135)*	*0-48 hr (n = 135)*	*72 hr–1 wk (n = 135)*
Stage 1					
Late decelerations	−0.07*	−0.04†	0.38*	0.38*	0.41*
Severe variable decelerations	−0.11*	−0.07*	−0.04	0.03	−0.02
Marked tachycardia	−0.04†	−0.05†	−0.02	−0.04	−0.02
Moderate variable decelerations	−0.04†	0.01	−0.02	−0.04	−0.02
Early decelerations	−0.05†	0.02	0.01	−0.09	−0.07
Normal baseline and variability	0.06‡	0.04†	−0.05	−0.15	−0.05
Stage 2					
Late decelerations	−0.14*	−0.06‡	0.32*	0.27‡	0.26‡
Early decelerations	0.04†	0.02	−0.14†	−0.09	−0.15

Only those correlations are shown for which at least one correlation had a $p < 0.05$.
*$p < 0.001$.
†$p < 0.05$.
‡$p < 0.01$.

Table IIIB. Correlations of selected labor variables and outcomes

	Electronic fetal heart monitoring		*Electronic fetal heart monitoring plus neurologic examination*		
				Neurologic examinations	
	Apgar score at 1 min (n = 2362)	*Apgar score at 5 min (n = 2362)*	*Convulsions (n = 135)*	*0-48 hr (n = 135)*	*72 hr–1 wk (n = 135)*
Meconium	−0.06*	−0.04†	0.23*	0.20†	0.22*
Duration of labor	−0.14‡	−0.06*	−0.04	0.13	0.06
Failure of labor to progress	−0.06*	−0.07‡	−0.07	0.04	0.01
Use of oxytocin	−0.12‡	−0.06*	−0.04	0.14	0.01
No. of scalp pH values	−0.11‡	−0.06*	0.05	0.13	0.13

Only those correlations are shown for which at least one correlation had a $p < 0.05$.
*$p < 0.01$.
†$p < 0.05$.
‡$p < 0.001$.

ance was accounted for, and this percent was further increased with electronic fetal heart monitoring. Specific abnormal electronic fetal heart rate patterns contributed significantly to outcome in the electronic fetal heart monitoring and electronic fetal heart monitoring–plus–neurologic examination groups; in the electronic fetal heart monitoring group, severe variable decelerations (stage 1) and late decelerations (stage 2); in the electronic fetal heart monitoring–plus–neurologic examination group, marked bradycardia (stage 2).

Table VI shows the regression analyses with the total score for the neurologic examination at 0 to 48 hours and at 72 hours to 1 week as the dependent variables. The neurologic examinations are much better accounted for when the specific electronic fetal heart patterns were used. Late deceleration in stage 1 was the major contributor to the percent variance accounted for in both neurologic examinations. The second neurologic examination should be a better predictor of the neurologic outcome of the baby than either Apgar score or first neurologic examination.

Table VII shows the regression analyses with use of forceps as the dependent variable. The intervention use of forceps was better accounted for than any other process in our analyses. In short, the model by which physicians act was very well described. In each subsample failure to progress was the leading contributor. Abnormal fetal heart patterns, either as a group or with individual patterns, contributed significantly in each subsample but varied in their ranking.

Table IV. Regression to Apgar score at 1 minute

Variable	*Standardized β*	*Significance of t*	*Multiple R and variance*
Auscultation			
No. of scalp pH values	0.09	0.00	Multiple R = 0.18
Duration of labor	0.07	0.00	Percent variance = 3.2
Failure of labor to progress	0.06	0.01	
Meconium	0.05	0.01	
Any heart rate abnormality	0.05	0.03	
Electronic fetal heart monitoring			
Late decelerations (2)	0.12	0.00	Multiple R = 0.24
Severe variable decelerations (1)	0.08	0.00	Percent variance = 5.8
Duration of labor	0.07	0.01	
Use of oxytocin	0.05	0.05	
Marked bradycardia (2)	0.05	0.02	
Meconium	0.04	0.05	
Severe variable decelerations (2)	0.04	0.05	
Auscultation plus neurologic examination			
Duration of labor	0.35	0.00	Multiple R = 0.49
Failure of labor to progress	0.23	0.01	Percent variance = 24.3
Electronic fetal heart monitoring plus neurologic examination			
Marked bradycardia (2)	0.25	0.01	Multiple R = 0.45
Late decelerations (2)	0.22	0.03	Percent variance = 20.0
Minimal variability (1)	0.22	0.02	

Table V. Regression to Apgar score at 5 minutes

Variable	*Standardized β*	*Significance of t*	*Multiple R and variance*
Auscultation			Multiple R = 0.04
No significant variables			Percent variance = 0.2
Electronic fetal heart monitoring			
Failure of labor to progress	0.06	0.01	Multiple R = 0.15
Severe variable decelerations (1)	0.05	0.02	Percent variance = 2.3
Late decelerations (2)	0.04	0.05	
Auscultation plus neurologic examination			
Duration of labor	0.24	0.02	Multiple R = 0.30
			Percent variance = 8.8
Electronic fetal heart monitoring plus neurologic examination			
Marked bradycardia (2)	0.23	0.02	Multiple R = 0.38
			Percent variance = 14.4

Table VIII shows the regression analyses with cesarean section as the dependent variable. This intervention is less well accounted for than is the use of forceps. The physicians appeared to operate on a clinical basis, mainly on the basis of variables other than fetal heart patterns.

Comment

The data from the Dublin trial constitute a large sample, with particular attention to the description and categorization of electronic fetal heart monitoring patterns during both first and second stages of labor. The obstetric intervention rate with use of either cesarean section or forceps was very low. It may be reasonable to assume that the relationship between fetal heart monitoring, including particular electronic fetal heart patterns, and neonatal outcome would be larger than might be expected in other studies if the intervention cesarean section is assumed to decrease abnormal neonatal outcome.

It is much easier to account for the interventions of the obstetricians than for the outcomes of the babies. It is clear that forceps were used in labors that were failing to progress, with additional contributions from variables that might reflect fetal distress, including abnormal fetal heart patterns. However, the several specific electronic fetal heart monitoring patterns that were predictive of use of forceps may result simply from an association of deviance in fetal heart patterns with failure of labor to progress. Our results do not necessarily indicate that the obstetrician considered all of these patterns to be helpful in making the decision to use forceps. The uses of cesarean section and forceps were based more on failure of labor to progress than on the fetal heart patterns.

Few specific fetal heart patterns were significantly

Table VI. Regression to first (0 to 48 hours) and second (72 hours to 1 week) neurologic examinations

Variables	*Standardized β*	*Significance of t*	*Multiple R and variance*
First neurologic examination (0-48 hr)			
Auscultation plus neurologic examination			
Any heart rate abnormality	0.24	0.01	Multiple R = 0.34
Meconium	0.23	0.01	Percent variance = 11.6
No. of scalp pH values	−0.22	0.03	
Electronic fetal heart monitoring plus neurologic examination			
Late decelerations (1)	0.36	0.00	Multiple R = 0.46 Percent variance = 21.3
Regression to second neurologic examination (72 hr–1 wk)			
Auscultation plus neurologic examination			
Any heart rate abnormality	0.20	0.05	Multiple R = 0.21 Percent variance = 4.3
Electronic fetal heart monitoring plus neurologic examination			
Late decelerations (1)	0.33	0.00	Multiple R = 0.51
Marked bradycardia (2)	0.30	0.00	Percent variance = 26.0

Table VII. Regression to use of forceps

Variable	*Standardized β*	*Significance of t*	*Multiple R and variance*
Auscultation			
Failure of labor to progress	0.83	0.00	Multiple R = 0.88
Any fetal heart abnormality	0.30	0.00	Percent variance = 77.8
Duration of labor	−0.05	0.00	
Use of oxytocin	0.03	0.01	
Electronic fetal heart monitoring			
Failure of labor to progress	0.73	0.00	Multiple R = 0.77
Marked bradycardia (2)	0.15	0.00	Percent variance = 59.0
Severe variable decelerations (2)	0.08	0.00	
Late decelerations (2)	0.08	0.00	
Moderate tachycardia with reduced variability (2)	0.06	0.00	
No. of scalp pH values	0.04	0.00	
Meconium	0.04	0.00	
Moderate variable decelerations (2)	0.04	0.00	
Late decelerations (1)	0.03	0.02	
Moderate variable decelerations (1)	0.03	0.04	
Auscultation plus neurologic examination			
Failure of labor to progress	0.77	0.00	Multiple R = 0.83
Any heart rate abnormality	0.54	0.00	Percent variance = 69.4
Duration of labor	−0.15	0.01	
Meconium	−0.12	0.02	
Electronic fetal heart monitoring plus neurologic examination			
Failure of labor to progress	0.77	0.00	Multiple R = 0.85
No. of scalp pH values	0.21	0.01	Percent variance = 72.9
Moderate variable decelerations (2)	0.21	0.00	
Late decelerations (2)	0.19	0.00	
Marked bradycardia (1)	0.17	0.00	
Meconium	−0.14	0.02	
Marked bradycardia (2)	0.14	0.02	
Severe variable decelerations (2)	0.13	0.01	
Minimal variability (1)	0.11	0.05	

associated with abnormal outcomes, either in the correlational analyses that used individual patterns (see Table IIIA) or in the regression analyses (Tables IV to VI), which included all ominous and suspicious patterns for stages 1 and 2 as defined by the obstetricians. We consider that Apgar scores and the neurologic evaluations represent quite different qualities about the neonate. Different configurations predict these different measures. The Apgar scores are probably broader measures registering different types of depression. Each improved across time: the Apgar score from 1 to 5 minutes and the neurologic evaluation from 0 to 48 hours to 72 hours to 1 week. Perhaps some of the confusion about the reliability of electronic fetal heart monitoring as a predictor of outcome is based on studies that used Apgar scores as a key outcome variable.

Table VIII. Regression to use of cesarean section

Variable	*Standardized β*	*Significance of t*	*Multiple R and variance*
Auscultation			
Failure of labor to progress	0.24	0.00	Multiple R = 0.34
Any heart rate abnormality	0.09	0.00	Percent variance = 11.7
Use of oxytocin	−0.09	0.00	
Meconium	0.08	0.00	
Electronic fetal heart monitoring			
Failure of labor to progress	0.16	0.00	Multiple R = 0.22
No. of scalp pH values	0.08	0.00	Percent variance = 4.8
Meconium	0.06	0.00	
Late decelerations (1)	0.06	0.00	
Late decelerations (2)	0.05	0.03	
Auscultation plus neurologic examination			
No. of scalp pH values	0.38	0.00	Multiple R = 0.68
Duration of labor	0.24	0.00	Percent variance = 46.0
Failure of labor to progress	0.23	0.00	
Meconium	0.23	0.00	
Any heart rate abnormality	0.18	0.02	
Electronic fetal heart monitoring plus neurologic examination			
Meconium	0.37	0.00	Multiple R = 0.47 Percent variance = 21.8

We consider neurologic evaluations at ages 0 to 48 hours and 72 hours to 1 week as better indicators of neurologic normality or abnormality than Apgar scores. The specific electronic fetal heart monitoring patterns of late decelerations (stage 1) and marked bradycardia (stage 2) do add significantly to the predictions to neurologic normality or abnormality (see Table VI). The percent variances accounted for are not large and perhaps not what the clinician might wish, but they indicate improvement from prediction that is based solely on other clinical variables.

In general, the Dublin trial perhaps has met criteria for excellence for randomized controlled trials better than most other electronic fetal heart monitoring[14] studies. Some have criticized the Dublin trial for the manner in which the fetal heart tracings were interpreted and the speed of the paper used in the electronic monitoring.[15] Systems have been proposed that incorporated accelerations, decelerations, and heart rate amplitude and variability in a single scoring.[16] Some categorizations in the Dublin trial did incorporate more than one variable, e.g., heart rate and variability. Only one categorization was permitted for each tracing. It is possible that another scoring system would modestly increase the percent variance accounted for. It is also possible that the use of trend analysis in contrast to pattern recognition for one or both stages of labor would increase the percent variance that was accounted for.

Another variable that was not scored with adequate distinction was the use of forceps. It would have been useful to distinguish between low forceps and mid forceps (high forceps reportedly were not used). That might have given more information about whether a modest increase in cesarean sections might have prevented the few forceps-related trauma cases (n = 3).

Fortunately, fetal conditions that are generally considered to be the most abnormal are very-low-frequency events. When there are no indicators of fetal stress, such as meconium, or no risk factors for fetal stress, such as use of oxytocin, prolonged labor, or failure to progress, then auscultation cannot be shown to be any less effective than electronic fetal heart monitoring. In this study, as in most others, auscultation was performed under a rigid protocol. However, when stress indicators appear, such as meconium, then specific, electronically monitored fetal heart patterns are additive in accounting for outcome.

We doubt that it is possible to increase more than modestly the accuracy of electronic fetal heart monitoring in predicting neurologic outcome by some combination of pattern analysis. Other variables add to the prediction, and they should be well heeded because there are so few variables that aid in making reasonable decisions. Furthermore, it is only in a small percentage of deliveries that the fetal heart patterns in combination with other variables can be used as justification for intervention. A technology that defines more than 40% of the heart rate patterns as deviant cannot possibly be accurate in predicting a very-low-frequency outcome such as neurologic abnormality. On the other hand, increased attention to the fetal heart rate and increased attention to the labor, as a result of the development of electronic fetal heart monitoring, may have significantly improved the outcome of labor and delivery.

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Cost-effectiveness of prenatal testing for *Chlamydia trachomatis*

Mary D. Nettleman, MD,[a] * and Thomas A. Bell, MD, MPH[b] **

Iowa City, Iowa, and Seattle, Washington

We investigated the cost-effectiveness of strategies for screening pregnant women for *Chlamydia trachomatis*. Screening was not cost-effective unless certain conditions were met. Direct antigen testing of all pregnant women would be cost-effective if the test cost <$6.30 or the prevalence of infection exceeded 6%. However, the positive predictive value of the test was only 51%. Culturing was not cost-effective until the prevalence of infection exceeded 14.8%. If a direct antigen test cost <$3.90 or prevalence exceeded 8.7%, direct antigen testing of all women and using culture to confirm positive direct antigen tests would be cost-effective. If a direct antigen test cost $8.00 and culture cost $25.00, the excess cost of performing a direct antigen test in all women and confirming positive results with culture would be $2.09 per pregnant woman. Screening all pregnant women for chlamydia is not cost-effective, but the excess cost is modest when direct antigen tests are used. (AM J OBSTET GYNECOL 1991;164:1289-94.)

Key words: *Chlamydia trachomatis*, decision analysis, prenatal care, cost-effectiveness

From the Department of Internal Medicine, University of Iowa College of Medicine,[a] and the Departments of Pediatrics and Epidemiology, University of Washington.[b]
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Reprint requests: Dr. Mary D. Nettleman, University of Iowa Hospitals C 41 GH, Iowa City, IA 52242.
**Dr. Nettleman is a Henry J. Kaiser Family Foundation Faculty Scholar in General Internal Medicine.*
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Diagnostic tests for detection of *Chlamydia trachomatis* have been shown to be cost-effective in nonpregnant women attending sexually transmitted diseases clinics who are not candidates for empiric therapy and in asymptomatic women at moderate risk for infection.[1, 2] The cost-effectiveness of screening pregnant women is more complicated because treatment options are limited, the sequelae of infection are more varied, and both mother and infant need therapy when a pregnant

Table I. Costs of conjunctivitis and outpatient pneumonia

Cost of conjunctivitis	
Physician visit	$ 35
Bacterial isolation, smear	$ 30
Direct antigen test	$ 8
Erythromycin	$ 7
Empiric treatment of mother	$ 10
Empiric treatment of male partner	$ 10
TOTAL	$100
Cost of outpatient pneumonia	
Two physician visits	$ 70
Direct antigen test	$ 8
Bacterial isolation, smear	$ 30
Respiratory syncytial virus test	$ 26
Blood profile	$ 19
Bordetella pertussis test	$ 20
One chest radiograph	$ 72
Erythromycin	$ 7
Empiric treatment of mother	$ 10
Empiric treatment of male partner	$ 10
TOTAL	$272

Table II. Cost of uncured chlamydial infection

Addverse outcome	*Cost*	*Probability*	*Weighted cost**
Neonatal conjunctivitis	$ 100	0.1900†	$19.00
Outpatient neonatal pneumonia	$ 272	0.0855†	$23.28
Inpatient neonatal pneumonia	$2500	0.0285†	$71.25
Outpatient salpingitis	$ 196	0.0840	$16.46
Inpatient salpingitis	$3071	0.0160	$49.14
Subsequent ectopic pregnancy	$4115	0.0070	$28.81
Outpatient epididymitis	$ 50	0.0400	$ 2.00
Inpatient epididymitis	$1876	0.0040	$ 7.50
TOTAL			$217.44

*Weighted cost is the cost of the adverse outcome multiplied by the probability of its occurrence.

†The probability of neonatal infections is adjusted for the proportion of women undergoing cesarean section.

woman is infected. We performed a decision analysis to determine the cost-effectiveness of various strategies for screening pregnant women.

Material and methods

Assumptions. Costs were based on local charges except where indicated. Although the terms *cost* and *charge* are used interchangeably, all monetary values were charges, which were chosen because they included the additional burden on third-party payers and the costs of health care resulting from disease or from screening programs. Potential complications of a chlamydial infection in a pregnant woman included conjunctivitis and pneumonia in her infant, epididymitis in her sexual partner, and her own risks of postpartum endometritis or salpingitis and ectopic pregnancy. The mean cost of neonatal conjunctivitis was assumed to be $100, and that of pneumonia caused by *C. trachomatis* in an infant outpatient, $272 (Table I). The cost of pneumonia in an inpatient was estimated to be $2500 from third-party fixed reimbursement for "viral pneumonia." A review of seven cases at the University of Iowa and five cases at the University of Washington yielded mean charges of $2465 and $4358, respectively, for *C. trachomatis* pneumonia in an inpatient. Therefore the effect of increasing the cost of pneumonia above $2500 was investigated by sensitivity analysis.

The costs of epididymitis were assumed to be $50 for outpatient therapy and $1876 for inpatient therapy.[3] The costs of outpatient and inpatient therapy for endometritis-salpingitis were $196 and $3071, respectively.[4] The cost of an ectopic pregnancy was $4115.[3,5] Adverse outcomes were assumed to occur in the first 2 years after an uncured infection; thus costs were not discounted.

The prevalence of chlamydial infection in pregnant women in the United States is approximately 5%.[6] Probabilities of complications were derived from the literature. The probabilities of conjunctivitis and pneumonia in infants born to infected mothers have been reported to be as high as 50% and 20%, respectively.[6-9] However, recent studies[10-12] have suggested that the occurrence of both events may be lower. We assumed that 25% of infants born to infected mothers developed conjunctivitis; 15% were assumed to develop pneumonia,[10,12] 75% of whom would need only outpatient therapy.[11] Twenty-four percent of women were assumed to have a cesarean delivery[13]; only vaginal births were assumed to result in neonatal infection.

Women who were not cured before delivery were assumed to have a 10% risk of postpartum endometritis-salpingitis,[14] with 16% of these cases necessitating hospitalization.[4] Seven percent of women in whom salpingitis developed were assumed to experience a subsequent ectopic pregnancy.[15,16] Each infected woman was assumed to have one infected partner. Male partners were assumed to remain untreated unless the woman were identified as infected or the man developed epididymitis. Approximately 4% of infected men were assumed to have epididymitis, 10% of whom required hospitalization.[3,17,18] The total mean cost of an uncured chlamydial infection in a woman was $217.44 (Table II).

Salpingitis leads to infertility in approximately 21% of nonpregnant women,[17,19] and the cost of an evaluation for infertility is approximately $2500.[5] The probability of infertility and the probability that women who have already been delivered of a child will seek evaluation for infertility have not been determined. Therefore infertility was not included in the analysis but was considered during sensitivity analysis.

The cost of screening included the costs of the tests, therapy if tests were positive, the side effects of therapy,

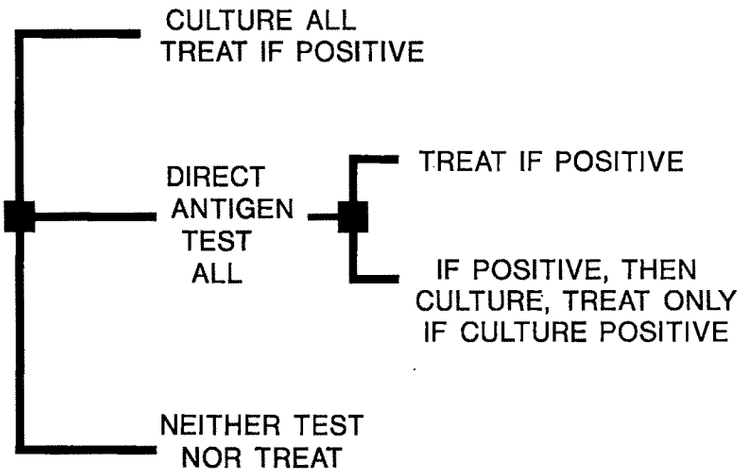


Fig. 1. Decision tree describing alternative screening strategies.

and empiric therapy of one male partner. The cost of a rapid direct antigen test was assumed to be $8, and the cost of treating a pregnant woman with erythromycin for 7 days was $10. Severe nausea and vomiting were assumed to require a visit to a physician at a cost of $35. Each woman whose test result was positive for *C. trachomatis* was assumed to have one sexual partner requiring empiric therapy ($10). We assumed that 3.3% of pregnant women would have severe nausea and vomiting from erythromycin and would not be cured.[11] For women who tolerated the medication, 92% would be cured with a single course of erythromycin.[11] Tests of cure after a course of therapy are not routinely recommended and were not included but were investigated during sensitivity analysis.[20]

Additional physician costs for examination were not added to the cost of routine screening because pelvic examination is routine in prenatal care. We assumed, however, that diagnosis of *C. trachomatis* conjunctivitis or pneumonia in an infant would prompt empiric therapy of the mother and one male partner.

The sensitivity of culture has been determined by comparing a single cervical specimen to multiple samples.[21,22] However, diagnostic tests for *C. trachomatis* are more sensitive in pregnant than in nonpregnant women.[23] Therefore we assumed that a single cell culture performed on a cervical swab had a sensitivity of 82% and a specificity of 100%.[23] A direct antigen test performed on a cervical swab was assumed to be 95% as sensitive and 96% as specific as culture.[23-25]

The analysis included only direct costs and excluded those related to transportation, emotional suffering, and time lost from work. We assumed that complications of *C. trachomatis* in infants would occur while women were still on maternity leave and that no wages would be lost. This may underestimate lost wages as a result of infant pneumonia, which is usually diagnosed after 6 weeks of age.[26] Approximately half of mothers with children under age 3 work, with a median income of $284 per week.[27] Sensitivity analysis was performed to determine if the addition of indirect costs to the cost of uncured infections affected the outcome of the analysis.

The effectiveness of a strategy was measured on a utility scale that ranged from 0 to 1, with 0 indicating the presence of uncured *C. trachomatis* in a pregnant woman and 1, the absence of *C. trachomatis* infection. Cost-effectiveness was defined as the total cost of a strategy divided by its effectiveness score (see Appendix).[1]

The following strategies were evaluated: (1) Neither test nor treat; (2) culture all pregnant women and treat if culture positive; (3) perform a direct antigen test in all women and treat if positive; (4) perform a direct antigen test in all women, confirm positive tests with culture, and treat only if the culture is positive (Fig. 1).

Results

Decision analysis. The most cost-effective strategy was neither to test nor to treat women for *C. trachomatis* (Table III). Testing all women with a direct antigen test was slightly less cost-effective, and testing all women with culture was least cost-effective. The strategy of testing all women with a direct antigen test and confirming positive results with culture had an intermediate cost-effectiveness value.

Detection of infection by culture had a specificity and positive predictive value of 100%. The positive predictive value of a direct antigen test used for this low-prevalence population was 51%, although the negative predictive value was high (Table III).

Cultures and direct antigen tests would detect 82% and 78% of all *C. trachomatis*, respectively. Confirmation

Table III. Cost-effectiveness and predictive values

Strategy	*Cost-effectiveness value*	*Positive predictive value*	*Negative predictive value*
Neither test nor treat	$11.77		
Direct antigen test all	$13.17	0.51	0.99
Culture all	$29.20	1.00	0.99
Culture only if direct antigen test is positive	$15.63	1.00	0.98

Table IV. Sensitivity analysis: breakpoints at which the three testing strategies become more cost-effective than not testing

	Direct antigen test all	*Direct test with culture confirmation*	*Culture all*
Cost of direct test	<$6.30	<$3.90	NA
Cost of culture	NA	Never	<$7.50
Infection prevalence	>6.1%	>8.7%	>14.8%
Cost of uncured infection	>$264	>$355	>$674
Probability of infant pneumonia	>22%	>36%	>87%
Probability of salpingitis in an untreated woman	>15%	>24%	>58%
Cost of outpatient pneumonia	>$815	>$1870	>$5700
Cost of inpatient pneumonia	>$4100	>$7300	>$18500

NA, Not applicable; *Never,* strategy is never more cost-effective than not testing at any value of the variable.

of positive direct antigen tests by culture would detect 64% of infections. For simplicity, the latter case assumes that the two tests are independent.

In 1988 there were approximately 3,941,000 births in the United States.[28] If each birth resulted from a single pregnancy, the national cost of screening all women with a direct antigen test would be approximately $8 million more than the cost of neither testing nor treating, representing an excess cost of $2.09 per pregnancy. If culture were performed in all pregnant women, $71 million in excess costs would be incurred ($17.94 per pregnancy). Finally, if direct antigen tests were performed in all pregnant women and positive results were confirmed with culture, the nation would incur $17 million in excess costs ($4.42 per pregnancy).

Sensitivity analysis. Sensitivity analysis was performed by varying each assumption over a range of possible values. The policy of testing and treating was less cost-effective than neither testing nor treating, unless certain conditions were satisfied. For example, if the cost of the direct antigen test were <$6.30 or the prevalence of infection in pregnant women were >6.1%, routine screening with the direct antigen test would be more cost-effective than neither testing nor treating (Table IV). Similarly, if the cost of the direct antigen test were <$3.90 or the prevalence of infection were >8.7%, using culture to confirm the direct antigen test would be more cost-effective than neither testing nor treating. Even using culture to test all women would be cost-effective if the prevalence of infection exceeded 14.8% or the cost per test fell below $7.50. Strategies involving the direct antigen test became cost-effective if the incidence of postpartum endometritis-salpingitis, neonatal pneumonia, or hospitalization for an infant with pneumonia were higher than we originally assumed. Specifically, changes that raised the mean cost of an uncured infection above $264, such as the addition of indirect costs, would make the use of the direct antigen test in all pregnant women cost-effective. For example, if the probability of pneumonia in the infant of an untreated, infected pregnant woman were >22% or the probability of conjunctivitis were >66%, direct antigen testing would become cost-effective.

If the cost of a test of cure had been included and 92% of women with failure of initial therapy were cured by a second course of erythromycin, the strategy of neither testing nor treating would remain the most cost-effective. The cesarean section rate did not affect the outcome of the analysis when allowed to range between 0% and 50%. The cure rate itself and the cost of treatment had no effect on the order of the strategies.

In addition, the sensitivity of culture did not affect the outcome of the analysis, even if it were assumed to be 100%. If culture-negative infected women were assumed to be so mildly infected as to have no complications, culture would remain less cost-effective than not testing. If indirect costs had been added and included 5 days off work for 50% of mothers whose children acquired pneumonia, direct antigen testing would be more cost-effective than neither testing nor treating.

The addition of costs for infertility did not affect the outcome of the analysis. Neither testing nor treating remained the most cost-effective strategy even if 21% of women with endometritis-salpingitis became infertile and 50% of them sought medical evaluation.

Comment

Testing pregnant women for diseases that can be transmitted transplacentally or perinatally is an important part of obstetric care. The advent of available, simple, and inexpensive screening tests has increased the possibility of routine prenatal screening for *C. trachomatis*.

Our analysis showed testing all pregnant women for *C. trachomatis* became cost-effective under certain conditions. If uniformly available for a charge of <$6.30 or with a population prevalence only 20% greater than what we assumed, routine screening with a direct antigen test would be more cost-effective than the current strategy of neither testing nor treating. If testing could be limited to subpopulations of women at moderately increased risk, direct antigen testing would become cost-effective. However, it is important for clinicians to realize that the positive predictive value of a direct antigen test in this population is only 51%. Thus half of women with positive tests, approximately 150,000 per year, would be treated unnecessarily. Conjugal relationships might be irreparably damaged from false-positive diagnoses. To minimize this damage, it would be important to interpret a positive direct antigen test only as an indication of increased probability of *C. trachomatis*, not as definitive evidence of infection.

Recent reports[29] indicate that the specificity of direct antigen testing may improve if all specimens are referred to central laboratories. Thus quality control may minimize false-positive diagnoses. In addition, bulk purchasing of direct antigen tests has lowered the cost per test to $4 in some instances. Such a test would be highly cost-effective.

To eliminate false-positive diagnoses, it would be cost-effective to use culture to confirm positive direct antigen test results if the charge for a direct antigen test were <$3.90 and the cost per culture were $25. If the cost of a direct antigen test remained at $8, $4.42 in excess charges would be incurred per pregnancy. More infected women would be missed by this strategy, but no uninfected women would receive treatment.

If culture were used to test all pregnant women, false-positive results would be eliminated and the maximum number of infected women would be identified for treatment. However, culture was not a cost-effective screening tool, and the charge for culture cannot yet be reduced to <$7.50.

When limited financial resources are available, decision analysis may guide providers to use strategies that maximize outcomes or minimize costs. However, an individual health care provider may choose a strategy that is not cost-effective, especially if the provider or patient does not have limited funds. Thus a physician may choose to culture out of concern for a patient's health and because the test has a high sensitivity, specificity, and positive predictive value. If all physicians adopted this strategy in caring for pregnant women, the cost of health care would increase by $17.94 per pregnancy.

Eradication of infection with *C. trachomatis* may have other benefits that are still under investigation. For example, infection has been linked to prematurity.[30,31] In addition, neonatal infection with *C. trachomatis* has been linked to chronic respiratory disease.[32,33] If either association is significant enough to increase the cost of uncured infection to >$264, then direct antigen testing would become cost-effective.

In summary, screening all pregnant women for infection with *C. trachomatis* would not result in a direct cost savings unless complications of uncured infection are shown to occur more frequently than assumed. However, the excess costs per pregnancy for either direct antigen testing or direct antigen testing with culture confirmation are modest and are justified by the morbidity of maternal and infant infection with *C. trachomatis*.

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Appendix: Sample calculations

Cost-effectiveness. The cost-effectiveness of a strategy was defined as its total cost divided by its total utility score.[1, 32] For example, if no pregnant women were tested for infection with *C. trachomatis,* the total cost of the strategy would be derived from the cost of complications of untreated infection ($217.44, Table II). Because 5% of women were assumed to be infected, the total cost per patient would be:

$$(\$217.44 \cdot 0.05) = \$10.87$$

Patients who were not infected would receive a utility score of 1. Patients who were infected would not be tested or identified and would receive a utility score of 0. Thus the utility score per patient would be:

$$(0 \cdot 0.05) + (1 \cdot 0.95) = 0.95$$

The cost-effectiveness value for this strategy would be:

$$\$10.87/0.95 = \$11.44$$

Note that better strategies with lower costs or higher utilities will have lower cost-effectiveness values. For strategies involving testing, the costs of the tests and treatment and the cure rate as a result of treatment are included.

Predictive value. Positive predictive value is defined as the probability of disease given a positive test. Thus the positive predictive value of direct antigen testing is the probability of true infection in a woman with a positive test. It may be calculated by dividing the number of true-positive results by the total number of positive results.[32]

For a direct antigen test with a sensitivity of 78% and specificity of 96% in a population with a 5% prevalence of infection, the proportion of results that would be true positive is:

$$\text{True positive} = \text{Sensitivity} \cdot \text{Prevalence} = 0.78 \cdot 0.05 = 0.039$$

Thus 3.9% of all women would have both a positive test and true infection. Note that only the population of truly infected women, 5% of the total population, may have true-positive results.

False-positive results occur when the test is positive but disease is not present. This is a failure in the specificity of the test. In the current example 95% of the population does not have disease and the number of false-positive results is:

$$\text{False positive} = (1 - \text{Specificity}) \cdot (1 - \text{Prevalence}) = 0.04 \cdot 0.95 = 0.038$$

Thus 3.8% of all women would be uninfected and would have a positive test.

The positive predictive value is:

$$\text{Positive predictive value} = \text{True positive}/(\text{True positive} + \text{False positive}) = 0.039/(0.039 + 0.038) = 0.51$$

Thus 51% of women with positive test results will truly be infected. Conversely, 49% of women with positive test results will not be infected and will be treated unnecessarily.

Intraamniotic detection of *Chlamydia trachomatis* deoxyribonucleic acid sequences by polymerase chain reaction

Chia C. Pao, PhD,[a] Shu-Min Kao, BS,[a] Han-Chow Wang, MD,[b] and Cagge C. Lee, MD[c]
Taipei, Taiwan, Republic of China

The presence of *Chlamydia trachomatis* in cervicovaginal cells and amniotic fluid of pregnant women was examined by culture and by polymerase chain reaction deoxyribonucleic acid amplification methods. Chlamydial deoxyribonucleic acid sequences were found in nine of 31 (29.0%) cervicovaginal cell specimens, five of which also were positive by culture method. Amniotic fluid specimens from two of the nine (22.2%) cervicovaginal chlamydial deoxyribonucleic acid–positive women and none from the remaining 22 cervicovaginal chlamydial deoxyribonucleic acid–negative women were found to contain chlamydial deoxyribonucleic acid. The results underscore the significance and frequency of intraamniotic chlamydial infection in women with urogenital chlamydial infections. (AM J OBSTET GYNECOL 1991;164: 1295-9.)

Key words: *Chlamydia trachomatis,* intraamniotic infection, polymerase chain reaction

Infection with *Chlamydia trachomatis* is the most common sexually transmitted disease in many parts of the world today.[1] In women, *C. trachomatis* may cause cervicitis and urethral syndrome in the lower urogenital tract and endometritis and salpingitis in the upper urogenital tract. These infections also can lead to sterility[2] and other complications during pregnancy.[3, 4]

Detection of *C. trachomatis* is very important in both diagnosis and treatment of chlamydial infection because a substantial proportion of women infected with *C. trachomatis* are asymptomatic. A diagnostic method that is more sensitive than the currently available culture and immunologic assays is needed to detect many asymptomatic and subclinical chlamydial infections. Deoxyribonucleic acid (DNA) hybridization has made it possible for early detection of chlamydial DNA in clinical specimens from both healthy individuals and infertile patients.[5, 6] The advent of polymerase chain reaction has provided us with a rapid and direct method that can amplify extremely small quantities of DNA, facilitating subsequent detection by conventional methods such as the DNA hybridization method.[7] Polymerase chain reaction has been used successfully in the detection of many microorganisms and we have reported its use in the identification of human cytomegalovirus, human papillomavirus, and *Mycobacterium tuberculosis*[8-10] in clinical specimens.

In this text we report the results of our studies on the detection of *C. trachomatis* DNA sequences by the enzymatic amplification technique, which suggests that *C. trachomatis* DNA sequences can be found in the amniotic fluid of a substantial proportion of women with urogenital chlamydial infection.

Material and methods

Source of specimens. Amniotic fluid was obtained by amniocentesis from 31 consecutive pregnant women who underwent the procedure for the purpose of genetic consultation. Cervicovaginal cells were collected from these patients by washing the cervix with 2 to 4 ml of phosphate buffer (20 mmol/L, pH 7.0) containing sucrose (200 mmol/L), bovine serum (5%), streptomycin (50 μg/ml), vancomycin (100 μg/ml), and nystatin (25 U/ml). None of the patients had known or diagnosed cervicovaginal chlamydial infection at the time of amniocentesis or cervicovaginal cell sampling. The average age of the patients was 25.2 years. Seven and nine of the women were pregnant for the first and second time, respectively, and the remainder were pregnant for the third time or more. Informed consents were obtained from all patients.

Chlamydial culture. Culture for chlamydia was performed with cycloheximide-treated McCoy cell monolayers described in detail elsewhere.[11] The reference strain of *C. trachomatis* (ATCC-VR 885) was obtained from American Type Culture Collection (Rockville, Md.).

Polymerase chain reaction. Cervicovaginal cells and cells in 1 ml of amniotic fluid were collected

From the Department of Biochemistry, Chang Gung Medical College,[a] Taipei Municipal Women's and Children's Hospital,[b] and Dr. Lee's Woman Hospital.[c]
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Reprint requests: Professor Chia C. Pao, Department of Biochemistry, Chang Gung Medical College, 199 Tun Hwa North Road, Taipei, Taiwan, Republic of China.
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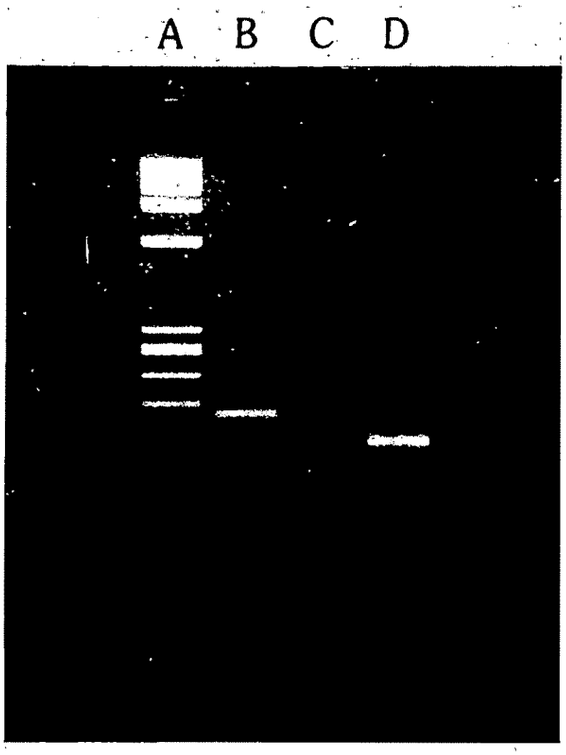


Fig. 1. Agarose gel electrophoresis of *C. trachomatis* DNA amplified with polymerase chain reaction. *Lane A* is restriction endonuclease *Hae* III–digested Phi-X 174 phage DNA used as size standards; the sizes of the DNA bands are (from top to bottom) 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, and 72 base pairs. *Lane B* shows amplification of DNA prepared from ATCC reference strain of chlamydia with 182 base pair DNA band indicating *C. trachomatis* DNA positivity. *Lane C* is negative control in which 50 ng of human DNA was amplified. *Lane D* is DNA shown in *lane B* after restriction endonuclease *Hha* I treatment. The 32 base pair DNA in *lane D* is too short to be visible clearly.

by centrifugation and washed in phosphate-buffered saline solution twice before they were resuspended in 250 μl of Tris hydrochloride buffer (10 mmol/L, pH 8.3) containing potassium chloride (50 mmol/L), magnesium chloride (2.5 mmol/L), gelatin (0.1 mg/ml), NP40 (0.45%), Tween 20 (0.45%), and proteinase K (500 μg/ml). The mixture was kept at 56° C for 1 hour, followed by 10 minutes at 95° C to inactivate the proteinase K. Ten microliters of the treated mixture was then used directly for the polymerase chain reaction. The nucleotide sequences of the two primers are (from the 5′-end to the 3′-end) AACTCAAAACCCTCTCATTCTCAA and AAACGTTCGTCCCAGGAAGAAGCC, and they cover a 182-base portion of a gene code for a major outer membrane protein of *C. trachomatis*.[12] The polymerase chain reaction procedures were those we have described earlier[9] with these modifications. Briefly, the 100 μl reaction mixture contains 10 mmol/L Tris hydrochloride buffer, pH 8.3; 50 mmol/L potassium chloride; 1.5 mmol/L magnesium chloride; 0.01% gelatin; 20 pmol each of the two primers; 2.5 nmol each of the four deoxyribonucleoside triphosphates; 1 unit of *Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.), and DNA from specimen, positive controls, or negative controls (see below). The temperature of the reaction mixture was first raised to 94° C for 20 seconds to denature the DNA, then brought down to 63° C for another 20 seconds to allow annealing of primers to putative target DNA sequences. The reaction mixture was then raised to 72° C for 1 minute to extend the DNA chain. This whole process was repeatd 32 times with a 10-minute incubation at 72° C at the end.

Establishment of *C. trachomatis* DNA positivity. Southern blot hybridization was performed on all polymerase chain reactions to enhance both the sensitivity and the specificity of the amplification process and to establish the *C. trachomatis* DNA positivity. For the Southern blot hybridization, 10 μl of amplified reaction mixture was fractionated by 2% agarose gel electrophoresis and transferred to nitrocellulose membrane filters. An internal oligonucleotide (AACATAAAAAGATATACAAAAATGGCTCTCTGCTTTATCG) derived from nucleotide sequences located inside the two primers on the *C. trachomatis* genome was labeled at its 5′-end with phosphorus 32 by polynucleotide kinase to 1 to 5×10^6 counts per minute per picomole and used as probe. Procedures for Southern blot hybridization have been described previously by others[13] and were modified for oligonucleotide probes as follows: After hybridization, the filters were washed sequentially in 2× SSC (1× SSC is 0.15 mol/L sodium chloride and 0.015 mol/L sodium citrate) and 0.5% sodium dodecyl sulfate twice for 10 minutes each at room temperature, then in 0.1× SSC and 0.5% sodium dodecyl sulfate three times for 20 minutes each at 55° C. Restriction endonucleases were purchased from either Boehringer Mannheim (Mannheim, Federal Republic of Germany) or BRL (Gaithersburg, Md.) and used according to manufacturers' recommendations.

Precautions against contamination and false-positive results in polymerase chain reaction. Because of the extraordinary amplification power of the polymerase chain reaction technique, a number of precautionary measures were taken to minimize any possible contamination during sampling and subsequent processing. Gloves were always worn during sampling and processing of specimens. Areas of skin were extensively cleaned before amniocentesis. Special steps also were taken to minimize sample-to-sample contamination and polymerase chain reaction product carryover to avoid false-positive results. These steps include aliquoting of

all reagents, physical separation of products and reagents before and after polymerase chain reaction, and meticulous laboratory techniques. Furthermore, all reagents were irradiated with ultraviolet light to inactivate any possibly contaminated double-stranded DNA that may have already existed in the reagents.[14] Fifty nanograms of human DNA or 10 ng of *Escherichia coli* DNA was used as a negative control that always yielded negative results in chlamydial DNA amplification reactions. Multiple reagent controls also were included in every polymerase chain reaction assay and always gave negative results. Repeated DNA amplification assays of the same specimens at different times or by different staff members produced the same results.

Results

The presence of chlamydial DNA is suggested by the appearance of the 182 base pair DNA after amplification (Fig. 1, lane B). The lower limit of detection of our method (i.e., DNA amplification by polymerase chain reaction followed by Southern blot hybridization with a ^{32}P-labeled internal oligonucleotide probe) is approximately a single chlamydial genome equivalent. The estimation of this sensitivity was reached by amplifying serial dilutions of chlamydia (ATCC-VR 885) of known infection titers in McCoy cells (data not shown). The level of detection is not adversely affected by the presence of as much as 50 ng of human or 10 ng of bacterial *E. coli* DNA in the specimens. DNA prepared from *Mycoplasma hominis, Mycoplasma pneumoniae,* human papillomaviruses (types 6, 11, 16, 18, and 33), human cytomegalovirus, and herpes simplex viruses (types I and II) do not cross amplify with the chlamydial primers used in this study (data not shown).

The authenticity of the amplified DNA and the specificity of the amplification reaction were evaluated and confirmed with two independent methods. First, the sizes of DNA after restriction endonuclease *Hha* I digestion of the amplified DNA, predicted from known DNA sequences and location of *Hha* I restriction site, were confirmed by the actual size produced after *Hha* I digestion. When the amplified chlamydial DNA was treated with restriction endonuclease *Hha* I, the original 182 base pair DNA disappeared and was replaced with new DNA of 150 and 32 base pairs (Fig. 1, lanes B and D). Second, Southern blot hybridization showed that the 182 base pair DNA, produced by amplifying DNA prepared from chlamydia-infected McCoy cells and from cervicovaginal cells and amniotic fluid of a patient with chlamydial infection, hybridized with the oligonucleotide probe specific for chlamydial DNA sequences (Fig. 2).

Chlamydial DNA sequences were found in cervicovaginal cell specimens of 9 of 31 (29.0%) pregnant women in this study (Table I). The cervicovaginal chlamydia positivity was confirmed by culture in only five of these nine chlamydial DNA–positive individuals. All five of the patients who had positive test results for chlamydia by both DNA and culture have clinical signs of mild mucopurulent cervicitis. Chlamydial DNA, on the other hand, was found in amniotic fluid specimens of two of nine (22.2%) women who had positive results for cervicovaginal chlamydial DNA. Of these two intraamniotic chlamydial DNA–positive individuals, one had positive results and one had negative results of both cervicovaginal cells and amniotic fluid cultures (Table



Fig. 2. Southern blot DNA hybridization of amplified *C. trachomatis* DNA with internal oligonucleotide probe. *Lane A* is amplification of DNA prepared from McCoy cells infected with ATCC reference strain of chlamydia, whereas *lane B* is from uninfected McCoy cells. *Lanes C and D* are amplifications of DNA prepared from cervicovaginal cells and amniotic fluid, respectively, of chlamydia-free patient. *Lanes E and F* are amplifications of DNA prepared from cervicovaginal cells and amniotic fluid, respectively, of patient with chlamydial infection. *Lane G* is primer-negative control. Exposure time for autoradiograph was 24 hours.

Table I. Prevalence of chlamydial DNA and culture in cervicovaginal cells and amniotic fluid

No. of patients	*Cervicovaginal cell*		*Amniotic fluid*	
	DNA	*Culture*	*DNA*	*Culture*
22	Negative	Negative	Negative	Negative
3	Positive	Negative	Negative	Negative
1	Positive	Negative	Positive	Negative
4	Positive	Positive	Negative	Negative
1	Positive	Positive	Positive	Positive

I). None of the amniotic fluid specimens from the remaining 22 cervicovaginal chlamydial DNA–negative women were found to contain chlamydial DNA. In a separate study the rate of positive chlamydial cultures was determined to be 11% among the general population (data not shown).

Comment

Genital chlamydial infections in women are important not only because they are frequent but also because the consequences can be serious. Among women with urogenital chlamydial infection, an estimated 10% can result in acute salpingitis. Subclinical endometrial infection can be rather frequent,[15] and a high proportion of women with involuntary infertility associated with bilateral tubal damage do not report a history suggestive of prior pelvic infection; therefore it is likely that the proportion of chlamydial infection with significant ascending but subclinical infection is much greater than the estimated 10%. A sensitive and reliable diagnostic method for *C. trachomatis* is important and needed for early diagnosis of subclinical chlamydial infections and for effective treatment.

Results reported here seem to have satisfied the two primary goals we set for this study: to examine the feasibility of using the polymerase chain reaction to detect chlamydia and to examine the prevalence of subclinical chlamydial infections. We have detected significantly more chlamydia by the polymerase chain reaction DNA amplification method than by the culture method (Table I), and the most likely reason for this difference is the higher sensitivity of the polymerase chain reaction method. False positivity probably did not contribute to the high DNA-positive rate because laboratory contaminations have been kept to the very minimum with various control measures, and the authenticity of the amplified chlamydial DNA was evaluated and confirmed by two independent methods. Although there is no direct evidence indicating that chlamydia can multiply in cells found in amniotic fluid, preliminary data from polymerase chain reaction amplification of ribonucleic acid suggested that chlamydia-specific messenger ribonucleic acid could be found in certain amniotic fluid specimens that are positive for chlamydial DNA (data not shown).

The pathways of intraamniotic chlamydial infection are not clear now, but the strong association between urogenital and intraamniotic chlamydial infections seems to suggest that ascension from the vagina and cervix may be the likely route through which chlamydia gains access to the amniotic cavity.

A number of microorganisms, including *C. trachomatis*[16] and others like *Gardnerella vaginalis, M. hominis,*[17-19] *Ureaplasma urealyticum,*[18, 19] human papillomavirus,[20] cytomegalovirus,[21] and human immunodeficiency virus,[22] have been identified or isolated from amniotic fluid or fetus. Some of them are known to be able to cause prenatal or congenital infections of the fetus.[21, 22] The presence of chlamydial DNA sequences by itself does not represent the presence of infectious chlamydia, but our results do suggest that the intraamniotic presence of chlamydia in women with urogenital chlamydial infection is probably not an infrequent event. The intraamniotic detection of chlamydia may suggest an in utero mechanism by which chlamydia can be transmitted before birth. However, the clinical significance and consequences of intraamniotic chlamydial infection need to be investigated further in light of the fact that none of the women with amniotic chlamydial infections in our study were reported to have chorioamnionitis, premature rupture of membranes, or preterm labor.

The results reported here underscore the frequency and importance of intraamniotic chlamydial infections in women with urogenital chlamydial infections. There is a definite need now for more detailed prospective studies on the relationship between intraamniotic chlamydial infections and infant morbidity.

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Birth weights of infants of black and white mothers without pregnancy complications

Thomas C. Hulsey, MSPH, ScD,[a] Abner H. Levkoff, MD,[a] and Greg R. Alexander, MPH, ScD[b]

Charleston, South Carolina, and Minneapolis, Minnesota

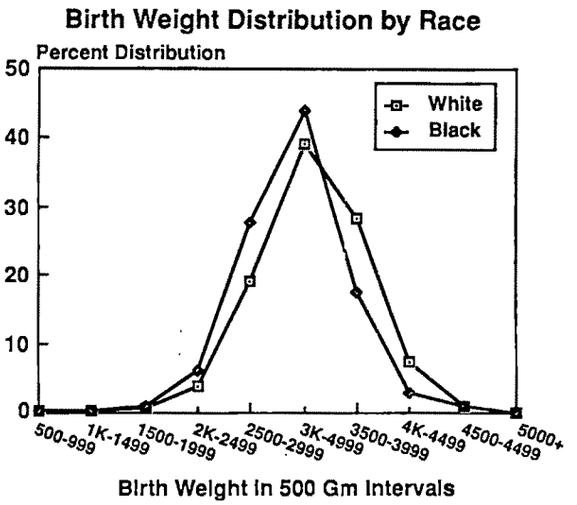
An analysis of 10,159 normal spontaneous vaginal deliveries was performed to examine racial differences in mean birth weight of infants whose mothers were without antepartum or intrapartum medical complications of pregnancy. The study was limited to black and white infants of low-income mothers who were inborn, singleton, and weighed ≥500 gm at birth. High-risk maternal transfer patients and patients with hypertension, toxemia, bacteriuria, pyelonephritis, renal failure, diabetes, anemia, polyhydramnios, oligohydramnios, prolapsed cord, vaginal bleeding, placenta previa, abruptio placentae, prolonged rupture of membranes, maternal fever on admission, amnionitis, sexually transmitted diseases, or fewer than five prenatal care visits were excluded. When statistically significant differences in demographic characteristics were controlled, black infants had an average birth weight 181 gm less than that of white infants. (AM J OBSTET GYNECOL 1991;164:1299-302.)

Key words: Birth weight, newborn, race, gestational age

Studies examining racial differences in mean birth weight have identified significant independent associations between birth weight, race, maternal demographic characteristics,[1, 2] and medical risk factors.[3] When differences in measured demographic characteristics or medical complications were controlled, significant racial differences in the mean birth weight remained.[4, 5] However, controlling for differences in the incidence of medical complications remains problematic, because the potential effects of racial differences in disease severity and compliance with therapy have not been considered.

To our knowledge no previous study of low-income mothers has attempted to control for the influence of medical complications of pregnancy on racial differences in mean birth weight by examining patients without such complications. A study so designed would also reduce the potential influence of unmeasured factors

From the Department of Pediatrics, Medical University of South Carolina,[a] and the Maternal and Child Health Program, School of Public Health, University of Minnesota.[b]
Received for publication December 19, 1989; revised October 12, 1990; accepted December 21, 1990.
Reprint requests: Thomas C. Hulsey, MSPH, ScD, Division of Neonatology, Department of Pediatrics, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425.
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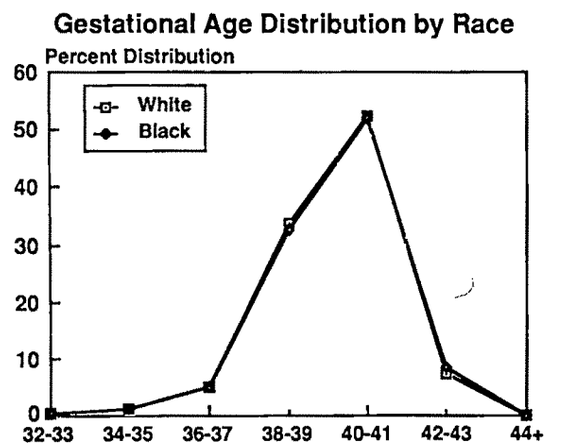


Fig. 1. Birth weight and gestational age percent distribution by race.

that are related to specific medical complications of pregnancy. More directly, do the observed racial differences in mean birth weight remain after the potential influences of disease are removed?

Material and methods

The Medical University Hospital is a regional perinatal center providing care for a predominantly low-income population, with 71.6% of deliveries during the past 7 years classified as indigent or Medicaid. Hospital records of all deliveries are routinely abstracted, and information from both maternal and newborn charts is entered into a computerized perinatal data base.[6] The validity and reliability of this data base are increased through extensive training of data clerks and regular checks for abstraction and coding errors.

Black and white mothers who were delivered between Jan. 1, 1981, and Dec. 31, 1987, were selected for the present study. Only singleton, live-born infants ≥500 gm delivered of black or white mothers who had at least five prenatal care visits were selected. To minimize the selection bias that may have resulted from high-risk maternal transfers, mothers who had been referred from another hospital were omitted from the study. In addition, many of the private patients who elected Medical University Hospital for delivery may have done so because of some previous poor pregnancy outcome. To minimize the latter bias, only nonprivate patient records were examined. Mothers who were delivered by cesarean section or vaginally after induction of labor also were excluded from study. In both instances the infant's birth weight could have been affected by an elective, iatrogenic termination of pregnancy, and it was unknown whether a trial of labor was attempted.

Table I. Comparison of maternal and infant characteristics by race

	Black	*White*	*p Value*
Single	69.2	32.9	0.0001
Mean height (inches)	64.1	64.1	0.95
Mean weight at delivery (lb)	163.7	160.1	0.0001
Education <12 yr (%)	45.3	60.7	0.0001
Primigravid (%)	33.8	34.9	0.26
Mean maternal age (yr)	22.7	22.2	0.0001
Low birth weight (%)	7.8	4.9	0.0001
Very low birth weight (%)	0.7	0.5	0.13
Mean gestational age (wk)	39.5	39.5	0.30
Mean head circumference (cm)	33.9	34.4	0.0001
Mean length (cm)	50.2	50.8	0.0001
Preterm (%)	4.0	3.9	0.85
Mean birth weight (gm)	3138	3322	0.0001

The maternal medical complications selected for exclusion were hypertension, toxemia, bacteriuria, pyelonephritis, renal failure, diabetes, anemia, polyhydramnios, oligohydramnios, prolapsed cord, vaginal bleeding, placenta previa, abruptio placentae, prolonged rupture of membranes, maternal fever on admission, amnionitis, and sexually transmitted diseases. Therefore the study population was limited to normal spontaneous vaginal deliveries free of major medical complications of pregnancy and homogeneous as to income, medical care, and predisposing perinatal risk. Of the total 22,949 births that occurred during the time period under investigation, 10,159 (44.3%) met all criteria for entry (6820 black and 3339 white).

Maternal demographic characteristics included mother's marital status, height, weight at delivery, age, gravidity, education, and race. Infant factors included birth weight, length, birth head circumference, and gestational age.[7]

Statistical analysis (Student's t test for continuous variables and χ^2 for categoric variables) first determined whether maternal demographic characteristics between

Table II. Regression analysis of demographic characteristics

Variable	*β**	*p Value*
Single marital status	−56.0	0.0001
Weight at delivery	4.7	0.0001
Education <12 yr	−9.06	0.36
Mother's age	2.4	0.02
Black race	−181.4	0.0001

Model: $R^2 = 0.14$; $p < 0.0001$; $N = 10{,}159$; F = 295.1; intercept = 2602.8.

*β-Coefficients reflect average change in birth weight (in grams) given a unit change of associated variable and with control for all other listed variables.

blacks and whites were significantly different. The effects of those factors that differed significantly between blacks and whites were simultaneously controlled by a linear regression model, and the adjusted mean birth weights were examined. The final model presents the average difference in mean birth weight in nonprivate, nonreferred, normal spontaneous vaginal deliveries between black and white women after statistically significant population differences were controlled.

Results

Maternal demographic and infant outcome characteristics are presented in Table I. The characteristics for which race was not statistically different were average maternal height at delivery and the proportion of primigravid women. Compared with black mothers, white mothers were more often married, weighed less at delivery, had completed fewer years of education, and were younger. The mean birth weight for black infants was 184 gm less than that for white infants. The reduced mean birth weight for black infants was accompanied by a significantly shorter mean length and smaller mean head circumference. The incidence of low birth weight was significantly higher in blacks. There were no significant racial differences in mean gestational age, incidence of preterm deliveries, or incidence of very-low-birth-weight births (birth weight <1500gm). Whereas the gestional age distribution was similar in both groups of infants, the birth weight distribution for white infants was shifted to the right (Fig. 1). The birth weight distribution shift between race groups was evident in both term and preterm infants (Fig. 2). The regression results in Table II suggest that maternal age and weight at delivery demonstrated a positive association with infant birth weight. Single marital status and maternal education <12 years was negatively associated with infant birth weight. After we simultaneously controlled for these factors, black infants continued to show an average birth weight 181.4 gm less than that of white infants.

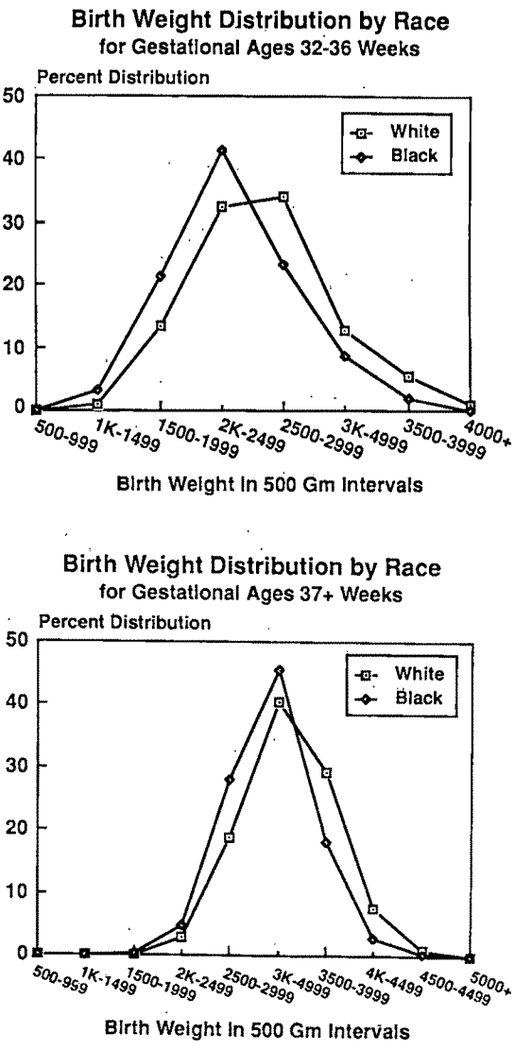


Fig. 2. Birth weight percent distribution by race and gestational age groups (32 to 36 and ≥37 weeks).

Comment

Previous epidemiologic reports have defined the discordance of mean birth weight between black and white infants to be approximately 200 gm, with black infants consistently weighing less than white infants. The cause of this mean weight difference, whether medical, environmental, or genetic, remains unanswered. The present study addresses the medical component by evaluating the birth weights of infants born by spontaneous vaginal delivery to low-income black and white mothers who had no medical complications of pregnancy.

This investigation suggests that, after attempts to eliminate the potential effects of medical complications and control for differences in maternal demographic characteristics, black infants continued to show a mean

birth weight that was significantly lower than that of white infants. While having a similar gestational age distribution, black infants were characterized by a statistically significant shorter average length and smaller average head circumference. Given the similarity of gestational maturity, a concomitant reduction in mean birth weight, mean length, and mean head circumference are consistent with symmetric growth reduction. The biologic basis of these differences remains unclear. Birth weight differences apparently were not due to a higher incidence of preterm gestational age among blacks or to the increased incidence of low birth weight in blacks. The low-birth-weight infants accounted for only a small portion of the 184 gm mean difference observed.

Several limitations should be addressed in comparing these two groups of mothers. Weight at delivery may not equate with nutritional status, nor years of schooling with quality of education, nor level of low income with depth of poverty. Gestational age at which prenatal care was initiated was not available and therefore was not assessed. Potential underreporting of medical complications and small cell sizes in the lower weight categories also may limit interpretation.

This investigation used the neonatal assessment to estimate gestational age. In this way neurologic and physical maturity were considered (rather than simply length of gestation). This allowed us to investigate whether reductions in birth weight were accompanied by differences in gestational maturity. The result would provide additional information as to the clinical implications of birth weight differences.

Given these limitations, however, this study suggests that in a moderately large population of low-income black and white parturients, without the known influence of medical complications of pregnancy and with controls for demographic differences, the mean size of black infants remained significantly smaller than that of white infants. Further, this difference was not accompanied by an equivalent difference in gestational maturity.

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Incidence of genital herpes simplex virus at the time of delivery in women with known risk factors

Patrick M. Catalano, MD, Alice O. Merritt, RN, and Philip B. Mead, MD
Burlington, Vermont

A total of 143 women with known risk factors for genital herpes simplex virus had cultures performed at the time of delivery. A total of 123 were without symptoms, of which three (2.4%) had positive herpes simplex virus cultures at the time of delivery. Fifteen women had lesions clinically consistent with genital herpes simplex virus at the time of delivery and five (33.3%) were culture positive. Five women had only prodromal symptoms of genital herpes simplex virus, but two of these (40%) had positive herpes simplex virus cultures from the site of previous lesions. Of the 10 women with positive herpes simplex virus cultures in this group of 143, no infant was delivered with evidence of neonatal herpes simplex virus infection, including two who had vaginal deliveries. The results of this study support the recommendations that in women without symptoms but with known risk factors for genital herpes simplex virus, a trial of vaginal delivery be allowed and that in women with either a lesion clinically consistent with genital herpes simplex virus or prodromal symptoms of genital herpes simplex virus a cesarean section be the mode of delivery. (AM J OBSTET GYNECOL 1991;164:1303-6.)

Key words: Maternal herpes simplex virus, parturition

The incidence of genital herpes simplex virus (HSV) infections has increased dramatically in the past decade.[1] Despite this fact, the management of genital HSV in obstetric patients at the time of delivery remains controversial. Protocols for obstetric management have as their goal the protection of the fetus from contact with HSV during labor and delivery. Although this mandates cesarean section in patients in labor with clinically active HSV lesions, the appropriate management of patients who might be shedding virus asymptomatically is less clear. Few studies have even assessed the incidence of asymptomatic HSV shedding during the intrapartum period. Harger[2] and Wittek[3] found a 1.9% to 2.3% rate of positive genital HSV cultures in pregnant women without symptoms who were at risk of genital HSV late in gestation. However, the only study that prospectively examined the incidence of positive genital HSV cultures at the time of delivery in women without symptoms but with a past history of genital HSV was that of Arvin et al.[4] who found an incidence of 0.96%.

Furthermore, asymptomatic shedding is unlikely to result in fetal infection. The observed risk of neonatal infection in 34 infants delivered vaginally to such women was zero in one study.[5] On the basis of this sample size, the 95% confidence interval for the neonatal infection rate is 0% to 8%. Because the neonatal infection rate will be the product of this low attack rate multiplied by the rate of asymptomatic genital shedding, confirmation of Arvin's[4] low incidence of shedding at delivery would suggest an extremely small neonatal infection risk in this setting. In accordance, this study was designed to determine the incidence of genital HSV shedding at the time of delivery in women with risk factors for such shedding.

From the Department of Obstetrics and Gynecology, University of Vermont College of Medicine.
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Reprint requests: Patrick M. Catalano, MD, Department of Reproductive Biology, MetroHealth Medical Center, Case Western Reserve University, 3395 Scranton Road, Cleveland, OH 44109.
6/1/27050

Material and methods

Subjects. This study was performed at the Medical Center Hospital of Vermont from 1984 through 1988. This hospital serves a population that is predominantly from the middle socioeconomic class. The study was approved by the Institutional Review Board at the University of Vermont, and informed consent was obtained from each participant. Women with either a past history of genital HSV, a sexual partner with genital HSV, or first episode of genital HSV infection during the index pregnancy were recruited antepartum to participate in this study.

Study design. Each woman received routine antepartum care from her primary obstetrician. The guidelines for the management of women at risk of genital HSV at the Medical Center Hospital of Vermont during the time of study consisted of weekly genital cultures for HSV during the last 4 weeks of pregnancy. Women whose most recent genital HSV cultures were negative and who were without symptoms on admission to the Labor and Delivery Unit were allowed to attempt vaginal delivery. Each patient had a genital and speculum examination for HSV at the onset of labor by the at-

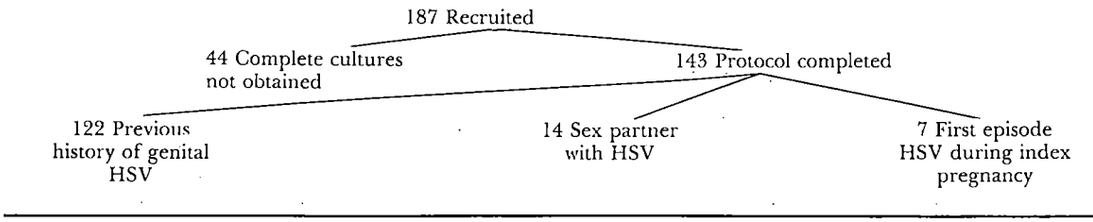


Fig. 1. Risk factors for genital HSV.

tending obstetrician or house officer. Women with a positive genital HSV culture within 1 week of delivery, a lesion clinically consistent with genital HSV lesion, or prodromal symptoms of genital HSV on admission to the Labor and Delivery Unit, were usually delivered by cesarean section. Individual intrapartum management decisions were made by the attending obstetrician.

For the purposes of this study, on admission to the Labor and Delivery Unit, a HSV culture was obtained from the woman's cervix and the usual site of lesion. A delivery record that described any lesions or symptoms of genital HSV and route of delivery was completed. The maternal obstetric record was reviewed and the number of episodes of genital HSV that were documented by the physical examination and positive HSV cultures obtained during the index pregnancy were noted. Last, each neonatal record was reviewed to determine whether HSV lesions were noted, cultures were obtained, and antiviral treatment was instituted.

HSV culture method. Cultures were obtained with a cotton-tipped swab. The swab was placed in a phosphate-buffered base transport medium. The medium contained distilled water, amphotericin B, gentamicin, fetal bovine serum, and sucrose. A total of 45% (64/143) of the cultures were performed at the Medical Center Hospital of Vermont. Aliquots of the transport medium were then inoculated into each of two tube cultures, one of which contained human fibroblasts and the other rabbit kidney cells. They were observed daily for evidence of characteristic cytopathic effects. A total of 55% (79/143) of the cultures were immediately placed in a −80° C freezer and later cultured at the University of Rochester virology laboratory because Dr. Richard Reichman, whose laboratory performed the HSV cultures, moved to Rochester during the time this study was in progress. The aliquots (0.2 ml) of the transport medium were then inoculated into each of two tube cultures of human diploid fibroblast cells and observed daily for evidence of characteristic cytopathic effects.

Results

Of the 187 women who were recruited for this study and who had a past history of genital HSV, a sexual partner with HSV, or clinical primary genital HSV infection during the index pregnancy, 143 completed the study. As shown in Fig. 1, 85% (122/143) had a previous history of genital HSV, 10% (14/143) had a sexual partner with only genital HSV, and 5% (7/143) had a first episode of genital HSV during the index pregnancy. The other 44 women had HSV cultures that were either lost because of a laboratory error or not obtained at the time of delivery.

Of the 136 patients who were at risk because of either a prior history of genital HSV or a partner with genital HSV, 27% (37/136) were documented to have a recurrent HSV lesion by physical examination during the index pregnancy—6 during the first trimester, 14 during the second trimester, and 17 during the third trimester. In these same patients, 29% (40/136) had a positive genital HSV culture during pregnancy—three during the first trimester, nine during the second trimester, and 28 during the third trimester. Hence, 45% (61/136) of patients with either recurrent genital HSV or a partner with genital HSV had either a genital HSV lesion or positive genital HSV culture during the index pregnancy.

Of the seven patients with a first episode of genital HSV infection during the index pregnancy, 57% (4/7) had a positive genital HSV culture—two in the second and two in the third trimester.

Incidence of genital HSV at the time of delivery. The results of the genital HSV cultures at the time of delivery are given in Table I. A total of 86% (123/143) of the patients were without symptoms at the time of delivery. Of the patients without symptoms, 2.4% (3/123) had a positive genital HSV culture.

A total of 10% (15/143) of the patients had a lesion clinically consistent with a genital HSV at the time of delivery, and 33% (5/15) had a positive genital HSV culture. Last, 4% (5/143) of the patients had only prodromal symptoms of genital HSV, and 40% (2/5) had a positive genital HSV culture.

Mode of delivery. Of the 123 women without symptoms 70% (86/123) had a vaginal delivery and 30% (37/123) were delivered of infants by cesarean section because of various indications. It is of note that 5% (6/123) of these women had a cesarean section solely on the basis of a history of genital HSV. In addition, two women were delivered of infants by cesarean section because of a positive genital HSV culture 1 to 3 days before delivery, although both had negative genital HSV cultures the day of delivery.

Two of the three women without symptoms but with a positive genital HSV culture the day of delivery were delivered of infants by cesarean section. One of these women had a labial lesion and positive HSV culture 1 week before delivery and the other women had a positive genital HSV culture 6 days before delivery. The third woman had a vaginal delivery, and it is of note that she had a negative genital HSV culture the day before delivery.

Of the 15 women with a question of a genital HSV lesion, 80% (12/15) were delivered of infants by cesarean section. One woman with a labial lesion had a precipitate vaginal delivery and a positive cervical HSV culture. The other two women were allowed vaginal deliveries and had negative genital HSV cultures.

In the five women with prodromal symptoms of HSV, four were delivered of infants by cesarean section because of their symptoms. One woman with a negative genital HSV culture had a vaginal delivery.

Neonatal outcome. All infants delivered of the 143 study subjects were healthy at the time of delivery and none had clinical evidence of neonatal HSV infection. Four infants were cultured for HSV and all cultures were negative.

Comment

The results of this study are comparable with the results of the study by Arvin et al.,[4] which was the only other study to prospectively examine the incidence of positive genital HSV cultures in an at-risk population at the time of delivery. The incidence of positive maternal genital HSV cultures in women without symptoms at delivery was 0.96% (3/311) in Arvin's study[4] in comparison with 2.4% (3/123) in our study.

However, Arvin's study[4] differed from ours in that the neonatal oropharynx, in addition to maternal genital HSV cultures, were obtained at the time of birth in their study. Two neonates, whose mothers had negative genital HSV cultures, had positive HSV cultures. Therefore the total incidence of asymptomatic shedding of genital HSV at the time of delivery was 1.4% (5/354). Neonatal cultures for HSV were not obtained routinely from our study population. In addition, Arvin's[4] patient population differed from ours in that they evaluated only women with a history of recurrent genital HSV infection, whereas 15% of our study population included women with either a partner with genital HSV (10%) or first episode of genital HSV (5%).

Although our study did not specifically address the question of whether antepartum maternal genital HSV cultures predicted which women without symptoms would have a positive genital HSV culture at the time of delivery, our results support the findings of Arvin[4] and Wittek[3] that antenatal genital HSV cultures are of little predictive value. The short mean duration of viral shedding with recurrent HSV late in pregnancy (3.6 ± 2.8 SD days as reported by Harger et al.[2]) and the fact that cultures for HSV have a sensitivity of <100%[6] may help to explain the poor predictive value of weekly genital HSV cultures in this population.

Table I. Incidence of genital HSV at delivery

Clinical status	*No. positive*	*No. cultured*	*%*
No. symptoms	3	123	2.4
HSV lesion	5	15	33.0
Prodromal symptoms	2	5	40.0

The incidence of positive HSV cultures in women with a lesion clinically consistent with genital HSV at delivery was between 20% to 30% in our study and that of Arvin.[4] These results support the generally accepted recommendation for cesarean section as the mode of delivery in these women. Moreover, we would also recommend cesarean section as the mode of delivery in those women with a history of recurrent genital HSV and prodromal symptoms of HSV. A total of 40% (2/5) of the women in this study and 20% (1/5) of the women in Arvin's study[4] had positive HSV cultures. Prodromal symptoms may be one of earliest manifestations of recurrent HSV in an at-risk population.

Optimal management of women without symptoms but with a history of genital HSV at the time of delivery is yet to be determined. However, our results support the current recommendations at the American College of Obstetricians and Gynecologists,[7] that a trial of vaginal delivery be allowed in such women. Furthermore, we recommend cesarean section as the mode of delivery in women with known risk factors for genital HSV and either a lesion consistent with genital HSV lesion or prodromal symptoms. The limitation of current management protocols to decrease the incidence of neonatal HSV infection, however, needs to be recognized. A recent national surveillance study for neonatal HSV infection by Stone et al.[8] showed that in a series of 184 cases of neonatal HSV infection, 78% of the mothers had no history of genital HSV infection. Furthermore, cesarean section before rupture of membranes failed to prevent neonatal HSV infection in 15 cases. Therefore future studies that examine the role of factors such as the titers of maternal neutralizing antibody, the development of accurate and rapid diagnostic tests at the time of delivery, and the potential use of antiviral agents during pregnancy are needed to decrease the incidence of neonatal HSV infection.

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The clinical utility of maternal body mass index in pregnancy

Honor M. Wolfe, MD,[a] Ivan E. Zador, PhD,[a] Thomas L. Gross, MD,[b] Susan S. Martier, MSSA,[a] and Robert J. Sokol, MD[a]

Detroit, Michigan, and Peoria, Illinois

To describe maternal body mass index and to compare the use of maternal weight and body mass index for risk assessment at the initial prenatal visit, 6270 gravid women who were consecutively delivered of infants were studied. Body mass index increased with advancing maternal age, parity, and advancing gestational age and was significantly greater in black women than in nonblack women. Risks for the development of adverse outcome associated with maternal obesity, including development of gestational diabetes, preeclampsia, fetal macrosomia, and shoulder dystocia, were comparably predicted by either maternal weight or body mass index >90th percentile. Maternal weight was as predictive of preeclampsia, macrosomia, and shoulder dystocia as was body mass index when these factors were analyzed as continuous variables, whereas increasing body mass index was more predictive of gestational diabetes. The prediction of factors associated with low maternal weights, small-for-gestational-age birth, prematurity, low birth weight, and perinatal death was equivalent for maternal weight and body mass index that was <10th percentile. This study indicates that in the initial risk assessment of outcomes related to maternal weight, the calculation of maternal body mass index offers no advantage over simply weighing the patient. This finding contrasts with results in nonpregnant women. (Am J Obstet Gynecol 1991;164:1306-10.)

Key words: Maternal weight, body mass index, perinatal risk, gestational diabetes

Many studies examining the effect of maternal size on pregnancy outcome relied on maternal weight (<100 and >200 pounds) to define risk categories at the initial prenatal visit.[1-3] More recently, risk assessment related to body size has been based on some measure of maternal weight for height.[4-6] Studies in the medical literature suggest that maternal body mass index, calculated as body weight (kilograms)/(height [meters])^2, is superior to weight alone for the prediction of morbidity and mortality in nonpregnant populations.[7,8] Because of the direct and continuous relationship of body mass index with outcome, its strong correlation with levels of adiposity, and its independence of height, a recent National Institutes of Health study[9] has recommended the use of body mass index as a measure of relative leanness in nonpregnant populations. In spite of the widespread use of maternal body mass index in the obstetric literature, we know of no studies to date that compare the relative validity of using maternal weight for height and maternal weight in the initial risk assessment for weight-associated outcomes in pregnancy.

Our purpose in this study was to describe body mass index and its determinants in pregnancy and to see

From the Department of Obstetrics and Gynecology, Hutzel Hospital/Wayne State University,[a] and the Department of Obstetrics and Gynecology, University of Illinois College of Medicine at Peoria.[b]
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Reprint requests: Honor M. Wolfe, MD, Department of Obstetrics and Gynecology, Hutzel Hospital, 4707 St. Antoine, Detroit, MI 48201.
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whether consideration of maternal weight for height (as calculated in the body mass index) leads to the better prediction of perinatal and maternal morbidity than does weight alone.

Material and methods

We selected women for study who were consecutively delivered of infants at Hutzel Hospital over a 30-month period and for whom antepartum and intrapartum records were available. Maternal and neonatal data were obtained from a computerized perinatal data base previously described.[10] Table I lists the variables obtained for each patient.

Body mass index was calculated from weight and height measured at the initial prenatal visit as weight (kilograms)/(height [meters])^2.

The effect of maternal characteristics on body mass index was examined by multiple regression with body mass index from the first prenatal visit as the dependent variable. Independent variables included maternal age, gestational age at the initial visit, race (coded as black vs nonblack), and maternal parity.

Maternal body mass index and maternal weight were then examined as continuous variables for their association with adverse outcomes associated with maternal size. To examine the relative predictive validity of maternal body mass index and weight in pregnancy, each factor was allowed to compete as an independent variable in separate discriminant function analyses against outcome variables previously associated with high and low maternal weight. Outcome (dependent) variables related to maternal obesity included gestational diabetes, preeclampsia, and the birth of a macrosomic infant (>4000 gm). Those variables associated with low maternal weight included the birth of a small-for-gestational-age infant, birth weight <10th percentile, very-low birth weight (<1500 gm), preterm birth (<37 weeks' gestation), and perinatal mortality. Maternal age, parity, race, marital status, gestational age at initial prenatal visit, and substance use were included as potentially confounding independent variables.

The relative risk for these adverse outcomes was then calculated for small maternal size (body mass index or maternal weight <10th percentile) and large maternal size (body mass index or maternal weight >90th percentile). Relative risks and the 95% confidence intervals[11] were calculated according to the method of Woolf.

Results

Table I summarizes the characteristics of the 6270 women studied. The group studied was predominantly black (79%) with a mean maternal age of 25 ± 6 years. The mean maternal body mass index at the first prenatal visit was 26.5 ± 6.5 kg/m^2 (64 inches, 155 pounds). The 10th percentiles for maternal weight and body mass index were 115 pounds and 20.1 kg/m^2, respectively; the 90th percentiles were 208 pounds and 35.2 kg/m^2.

Table I. Characteristics of 6270 women studied

Factor	*Mean ± SD*	*Range*
Age (yr)	25.1 ± 6.0	12-52
Gravidity (yr)	3.0 ± 1.9	1-16
Parity	1.1 ± 1.4	0-11
Race (%)		
Black	79.0	
Other	21.0	
Marital status (%)		
Married	34.0	
Nonmarried	66.0	
Gestational age (wk)		
First prenatal visit	19.3 ± 8.6	5-42
Delivery	39.1 ± 2.3	20-44
Prepregnancy weight (lb)*	142.5 ± 38.0	62-411
Weight first visit (lb)	155.3 ± 40.6	78-414
Maternal height (in)	64.2 ± 2.8	48-75
Body mass index (kg/m^2)		
Prepregnancy	24.3 ± 6.1	13.2-68.5
First visit	26.5 ± 6.5	13.2-69.0
Medical complications (%)		
History of dibetes	2.8	
History of hypertension	3.7	
Substance use-abuse (%)		
Tobacco	23.7	
Marijuana	7.4	
Narcotic	6.5	
Alcohol abuse	4.1	

*Patient recall.

Results of the multiple regression for determinants of maternal body mass index are summarized in Table II. When considered individually (step 0), body mass index was found to increase with increasing maternal age, parity, and advancing gestational age. Black women had a significantly greater body mass index than did nonblacks (26.8 vs 25.5 kg/m^2, respectively). This was true in spite of the fact that, on average, black mothers were significantly younger than nonblack mothers (24.2 vs 27.6 years). Body mass index decreased with a maternal history of alcohol, narcotics, or tobacco use. When considered jointly (at the final step), all maternal factors remained significant, with a total of 9.7% of the variance in body mass index explained by these variables.

When maternal body mass index and weight were examined as continuous variables, as shown in Table III, discriminant analysis revealed that of the outcome variables associated with maternal obesity, only gestational diabetes mellitus was better predicted by increasing maternal body mass index than by weight. Even

Table II. Summary of multiple regression for determinants of maternal body mass index at initial prenatal visit

Variable	Step 0		Final step		Explained variance (%)
	F test	p Value	F test	p Value	
Maternal age	158.9	0.001	183.2	0.001	2.9
Gestational age	126.8	0.001	185.2	0.001	3.4
Maternal race	37.1	0.001	84.0	0.001	1.5
Narcotic use	24.2	0.001	63.7	0.001	1.1
Maternal parity	150.9	0.001	16.8	0.001	0.3
Alcohol use	11.8	0.001	15.8	0.001	0.1
Tobacco use	5.5	0.02	7.3	0.01	0.1
Marital status	0.9	NS	8.0	0.01	0.1

df 1 to 5280; total R^2 = 9.7%.

Table III. Summary of discriminant analysis for determinants of gestational diabetes

Variable	Step 0		Final step		Explained variance (%)
	F test	p Value	F test	p Value	
Body mass index*	160.4	0.001	160.4	0.001	3.0
Maternal age	119.9	0.001	81.5	0.001	1.5
Narcotic use	3.6	NS	5.3	0.02	0.1
Maternal weight*	141.1	0.001	0.2	NS	
Maternal parity	31.9	0.001	0.3	NS	
Maternal race	4.2	0.04	1.1	NS	

df 1 to 5280; total R^2 = 4.6%.
*At first prenatal visit.

when maternal weight was forced into the model at level 1, body mass index significantly improved the prediction of gestational diabetes. Indeed, increasing maternal body mass index was more predictive of the development of gestational diabetes than of advancing maternal age. Macrosomia, shoulder dystocia, and preeclampsia were comparably predicted by increasing maternal weight and body mass index, which were considered as continuous variables.

The study also examined outcomes associated with low maternal weight. Discriminant function analysis demonstrated that the birth of small-for-gestational-age infants was most closely associated with narcotic use; decreasing maternal weight explained minimal additional variance.

In this study, perinatal mortality, very low birth weight, and preterm births were not predicted by either maternal body mass index or weight, when these were considered as continuous variables.

Relative risks for outcomes associated with maternal obesity were comparable for maternal weight or body mass index >90th percentile (Table IV). Similarly, relative risks for adverse outcomes associated with low maternal weight were equal for maternal weight or body mass index <10th percentile (Table V).

Comment

This is the first study, of which we are aware, that describes determinants of maternal body mass index and compares maternal body mass index with maternal body weight for the strength of their associations with perinatal outcomes. Maternal factors, including age, race, parity, substance use, and gestational age, all were significantly related to body mass index and explained approximately 10% of its variance. Although diabetes mellitus was more strongly associated with maternal body mass index than with maternal weight, four of the five outcomes significantly related to maternal size (macrosomia, preeclampsia, and infants who were either small or large for gestational age) were more strongly related to weight than to body mass index. In contrast to previous data in nonpregnant populations, this study suggests that, in the pregnant patient, body weight is comparable to body mass index for the prediction of key adverse, weight-associated outcomes.

This study was not designed to examine the well-established association between maternal size or weight gain and perinatal or maternal outcome. Rather, data were analyzed to address the more basic question of whether this relationship might be better expressed by the recent trend in the medical and obstetric literature

Table IV. Comparison of relative risk for occurrence of outcomes associated with maternal obesity for body mass index or weight >90th percentile

Outcome	*Maternal size assessment*	*Relative risk*	*95% Confidence interval*
Gestational diabetes	Body mass index >90th percentile	5.31	3.83-7.34
	Weight >90th percentile	4.18	3.03-5.75
Preeclampsia	Body mass index >90th percentile	2.26	1.71-2.99
	Weight >90th percentile	2.49	1.93-3.21
Macrosomic infant	Body mass index >90th percentile	2.54	1.94-3.33
	Weight >90th percentile	2.77	2.16-3.55
Large-for-gestational-age infant	Body mass index >90th percentile	2.05	1.62-2.59
	Weight >90th percentile	2.49	2.00-3.09
Shoulder dystocia	Body mass index >90th percentile	1.80	0.89-3.66
	Weight >90th percentile	3.50	0.14-3.19

Table V. Comparison of relative risk for outcomes associated with low maternal weight for body mass index or weight <10th percentile

Outcome	*Maternal size assessment*	*Relative risk*	*95% Confidence interval*
Small-for-gestational-age infant	Body mass index <10th percentile	2.07	1.58-2.72
	Weight <10th percentile	2.07	1.59-2.70
Premature infant	Body mass index <10th percentile	1.64	1.24-2.17
	Weight <10th percentile	1.83	1.41-2.38
Low-birth-weight infant	Body mass index <10th percentile	2.09	1.64-2.66
	Weight <10th percentile	2.31	1.84-2.89
NICU admission*	Body mass index <10th percentile	1.25	0.88-1.78
	Weight <10th percentile	1.42	1.01-1.98

*Neonatal intensive care unit admission.

toward the use of body mass index. We found that body mass index was superior to maternal weight only for identifying risk for gestational diabetes. It is not surprising that diabetes correlates better with body mass index, because insulin resistance is more a function of fat content than of absolute weight. This may reflect the fact that body mass index is more sensitive than weight to obesity in shorter women. Thus, for example, a woman who is 5 feet tall and weighs 180 pounds has a body mass index in the "obese" range (35.3 kg/m^2), although she does not meet the weight criteria for obesity.

Body mass index is used to define relative leanness and weight-associated outcomes in nonpregnant populations. It is not clear why this did not appear to apply to pregnant women. This study was performed with a sample of indigent, black, inner-city gravid women. The mean prepregnancy body mass index in this study (24.3 kg/m^2) was higher than the "ideal" body mass index for nonpregnant populations (22.5 kg/m^2).[9] Therefore our results may not be entirely applicable to other populations. In addition, although body mass index correlates with the percent of fat, body mass index does not distinguish between various body components such as muscle and fat. Because our patients sought prenatal care at a relatively late gestational age (20 weeks), this study cannot address the relative utility of maternal body mass index and weight in the first trimester.

Although in individual cases maternal body mass index may be helpful in defining risk as it is related to maternal size (particularly among women at either extreme of height), body mass index and weight appear to be of comparable use in large studies. Whereas much remains to be defined with respect to maternal leanness and perinatal outcome, this study suggests that the simple act of weighing the patient constitutes an important part of prenatal care and is adequate for the identification of most perinatal risk factors related to maternal size.

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Complexes of activated protein C with α_1-antitrypsin in normal pregnancy and in severe preeclampsia

Francisco España, PhD,[a] Juan Gilabert, MD, PhD,[b] Justo Aznar, MD, PhD,[c] Amparo Estellés, MD, PhD,[a] Takao Kobayashi, MD,[c] and John H. Griffin, PhD[d]
Valencia, Spain, and La Jolla, California

Protein C is a vitamin K–dependent regulator of blood coagulation. Activated protein C is regulated in plasma in large part by two inhibitors, protein C inhibitor and α_1-antitrypsin. Complexes of activated protein C with both inhibitors in plasma samples from subjects with normal or pathologic pregnancy were measured. In normal pregnancy we observed a progressive and significant increase in activated protein C/α_1-antitrypsin complex levels, from 9 ± 3 ng/ml in the first trimester to 16 ± 3 ng/ml in the third trimester, as well as an increase in α_1-antitrypsin plasma levels. In severe preeclampsia, but not in chronic hypertension with superimposed severe preeclampsia, there was a greater increase in activated protein C/α_1-antitrypsin levels (25 ± 10 ng/ml) ($p < 0.001$) and a decrease in protein C and protein C inhibitor levels as compared with normal pregnant women at similar gestational ages. These data show an increase in the activation of the protein C pathway in both normal and pathologic pregnancy and provide evidence for an enhancement of thrombin generation in severe preeclampsia compared with chronic hypertension with superimposed severe preeclampsia. (AM J OBSTET GYNECOL 1991;164:1310-6.)

Key words: Protein C complexes, α_1-antitrypsin, pregnancy, preeclampsia, chronic hypertension

Alterations in hemostasis during normal gestation are widely known, and some obstetric complications, for example, preeclampsia, may increase the hypercoagulability state observed in pregnancy. Thus increased levels of coagulation factors[1, 2] and reduced fibrinolysis[3] have been demonstrated in preeclampsia. Disseminated intravascular coagulation has also been demonstrated in some cases of severe preeclampsia and eclampsia,[4] the action of the enzyme thrombin on fibrinogen being crucial in this process.

Activated protein C, the product of thrombin's action on protein C in the presence of endothelial cell thrombomodulin, together with its cofactor, protein S, regulates blood clotting by inhibiting the clotting cascade at the levels of factors Va and VIIIa.[5, 6] Activated protein C is regulated in plasma by two inhibitors, protein C inhibitor[7] and α_1-antitrypsin,[8] and complexes of activated protein C with both inhibitors have been reported to be circulating in vivo.[8, 9] The importance of the protein C anticoagulant system is illustrated by the fact that newborns with homozygous protein C deficiency suffer severe and generalized thrombotic disease,[10] and

From the Research Center[a] and the Departments of Obstetrics and Gynecology[b] and Clinical Pathology,[c] Hospital "La Fe," and the Committee on Vascular Biology and the Department of Molecular and Experimental Medicine, Research Institute of Scripps Clinic.[d]
Supported by a research grant from Fondo de Investigaciones Sanitarias de la Seguridad Social (90/0586), Spain.
Received for publication April 25, 1990; revised October 31, 1990; accepted December 21, 1990.
Reprint requests: Francisco España, PhD, Hospital "La Fe," Centro de Investigación, Av. Campanar 21, 46009 Valencia, Spain.
6/1/27580

Table I. Clinical and analytic data in normal and pathologic pregnancies

	Normal pregnancy, third trimester (n = 27)	*Preeclampsia* (n = 14)	*Chronic hypertension with superimposed severe preeclampsia* (n = 7)
Age (yr)	29 ± 4 (18-41)	28 ± 5 (21-37)	35 ± 4 (28-41)
Nulliparous women	15	12	1
Gestational age at sampling (wk)	36 ± 3 (29-41)	35 ± 6 (28-40)	33 ± 3 (28-37)
Birth weight (gm)	3245 ± 477	2277 ± 1210	2133 ± 1097
Placental weight (gm)	646 ± 126	514 ± 156	547 ± 157
Perinatal deaths	0	2	2
Hematocrit (%)	39 ± 3 (32-42)	34 ± 6 (26-41)	35 ± 3 (30-36)
Total bilirubin (mg/dl)	0.2 ± 0.1 (0.1-0.4)	0.4 ± 0.1 (0.1-0.6)	0.3 ± 0.1 (0.1-0.5)
Lactic dehydrogenase (IU/L)	137 ± 10 (100-178)	176 ± 52 (106-209)	177 ± 29 (138-215)
Aspartate aminotransferase (IU/L)	14 ± 8 (11-23)	15 ± 6 (9-23)	17 ± 6 (8-23)
Alanine aminotransferase (IU/L)	18 ± 8 (10-29)	21 ± 16 (8-56)	32 ± 21 (5-67)
Creatinine clearance (ml/min)	126 ± 10 (95-150)	90 ± 25 (60-119)	94 ± 37 (61-167)
Uric acid (mg/dl)	5 ± 1 (3-6)	6 ± 2 (2-7)	6 ± 1 (5-7)

Values are mean ± SD and range (shown in parentheses).

heterozygous protein C or protein S deficiency in some patients and their families is associated with venous thromboembolism.[11, 12] Recent evidence suggests that alterations in protein C pathway may be involved in thrombotic complications associated with normal and pathologic pregnancy. Functional and immunologic levels of protein S are decreased in normal pregnancy,[13, 14] and reduced levels of both protein C and protein S have been described in patients with severe preeclampsia.[14]

The purpose of this investigation was to gain qualitative and quantitative information regarding the activation and inactivation of protein C and its distribution between its two known inhibitors during normal and pathologic pregnancy, as a measure of the thrombin activity. In this study we measured protein C, protein C inhibitor, α_1-antitrypsin, and complexes of activated protein C with its two inhibitors during pregnancy and in patients with preeclampsia, and we report that activated protein C/inhibitor complexes are significantly increased during pregnancy and that patients with severe preeclampsia have activated protein C/α_1-antitrypsin complex levels twice as high as those in both chronic hypertension with superimposed severe preeclampsia and gestational age–matched controls.

Material and methods

Clinical groups

Control group. The control group for the pregnant groups comprised 20 normal nonpregnant women, aged 18 to 40 years (29 ± 5), who were not taking oral contraceptives and had no history of thromboembolic disease.

Normal pregnant group. Seventy-eight normal pregnant women, ranging from 18 to 41 years of age (29 ± 4), were included in the study. These women were grouped according to gestational age in first (n = 18), second (n = 33), and third (n = 27) trimester. No complications were noted during the pregnancies. They were nonsmokers and did not receive any type of medication except vitamin complexes.

Severe preeclampsia group. This group comprised 14 patients, aged 21 to 37 years (28 ± 5), with no previous history of hypertension but in whom a preeclamptic state developed during the third trimester of pregnancy. Criteria for severe preeclampsia, according to the Committee on Terminology of the American College of Obstetricians and Gynecologists,[15] were blood pressure ≥160/110 mm Hg and proteinuria > 1 gm/L. Both parameters were checked and were abnormal on at least two random occasions, 6 hours apart.

Chronic hypertension with superimposed severe preeclampsia. This group was made up of seven patients aged 28 to 41 years (35 ± 4) with hypertension developing before the twentieth week of pregnancy and later development of a severe preeclamptic state. In all these patients hypertension remained for at least 40 days after delivery.

Table I shows some clinical and analytic data on these groups. None of the patients in the preeclampsia groups showed substantial alterations in the levels of hepatic enzymes. The control group for patients with

preeclampsia was the group of normal pregnant women in the third trimester of pregnancy. Normal plasma was pooled from 24 apparently healthy men with no history of bleeding or thrombosis. Informed consent was obtained from all patients and control subjects before blood collection.

Venous blood samples were obtained from the cubital vein in 1:9 (vol/vol) 0.11 mol/L trisodium citrate with or without 50 mmol/L benzamidine (Sigma Chemical Co., St. Louis), and plasma samples were stored in aliquots at −70° C.

Assays. Protein C antigen and activity[16] and protein C inhibitor antigen and activity[17] were assayed as previously described. Activated protein C/protein C inhibitor[17] and activated protein C/α_1-antitrypsin[18] complexes were determined by sandwich enzyme-linked immunosorbent assay as described before. Briefly, activated protein C complexes were assayed by coating plates with a monoclonal anti–protein C antibody, and complexes of activated protein C bound to the plates were detected with peroxidase-labeled rabbit anti–protein C inhibitor and anti-α_1-antitrypsin antibodies, respectively. The detection limit for activated protein C/protein C inhibitor and activated protein C/α_1-antitrypsin assays was 3 and 1 ng/ml, respectively. Calibration curves were constructed with known amounts of purified preformed activated protein C complexes. Blood for activated protein C complex assays was anticoagulated with citrate containing 50 mmol/L benzamidine.

α_1-Antitrypsin was measured by rocket immunoelectrophoresis with a commercial rabbit antiserum against human α_1-antitrypsin (Sigma) in 1% agarose in Tris-glycine buffer (0.038 mol/L Tris, 0.1 mol/L glycine, 2.1 mmol/L ethylenediaminetetraacetate, and 0.02% sodium azide, pH 8.7) with 0.46% (vol/vol) antiserum. Plasma was diluted 1:200 in Tris-glycine buffer, and 12 μl of the diluted plasma was applied to sample wells. Electrophoresis at 8 mA (15 v/cm) was carried out overnight at room temperature. The gels were washed twice for 2 hours in 0.5 mol/L saline solution and then twice in distilled water; immunoprecipitates were visualized with Coomassie Brilliant Blue R-250.

Because of the normal distribution of data, changes in the levels of activated protein C/α_1-antitrypsin complex, protein C inhibitor, α_1-antitrypsin, and protein C throughout pregnancy were subjected to computerized analysis of variance. The level of activated protein C/protein C inhibitor complex in normal and pathologic pregnancy was compared with the χ^2 test, and Yates' correction was applied when necessary. The Student *t* test was used for comparison between pathologic pregnancy groups and the third trimester of pregnancy.

Results

The level of activated protein C/α_1-antitrypsin complex in plasma samples of pregnant women at different gestational ages was compared with that of nonpregnant control subjects (Fig. 1). The pregnant women showed mean complex levels of 9 ± 4 ng/ml (mean ± SD) (first trimester), 12 ± 3 ng/ml (second trimester), and 16 ± 3 ng/ml (third trimester), whereas the nonpregnant women had a mean complex level of 8 ± 3 ng/ml, with a range of 3 to 14 ng/ml; the difference between nonpregnant and pregnant women in the second and third trimesters of pregnancy was significant at the $p < 0.001$ level. There was a good correlation between activated protein C/α_1-antitrypsin complex levels and weeks of gestation ($r = 0.636$, $p < 0.001$). The severe preeclampsia group showed a marked increase in the level of activated protein C/α_1-antitrypsin complex (29 ± 9 ng/ml, $p < 0.001$) compared with that of normal pregnant women in the third trimester of pregnancy. In contrast, the group of pregnant women with chronic hypertension and superimposed severe preeclampsia had an activated protein C/α_1-antitrypsin complex level (15 ± 6 ng/ml) that was similar to that of the control group of women in the third trimester of pregnancy.

The level of activated protein C/protein C inhibitor complexes in all groups is seen in Fig. 2. All plasma samples from nonpregnant women had levels below the detection limit of the assay used (<3 ng/ml). Of 78 normal pregnant women studied, detectable activated protein C/protein C inhibitor complex levels were found in only four women. Seven of the 14 patients with severe preeclampsia had detectable levels ranging between 7 and 21 ng/ml (median, 13 ng/ml), and only one of the seven patients with chronic hypertension with superimposed severe preeclampsia had a detectable level of 20 ng/ml. The difference between the levels in normal pregnant women and those with severe preeclampsia was significant at $p < 0.001$. However, no significant differences were observed between the levels in normal pregnant women and those found in chronic hypertension with superimposed severe preeclampsia. In patients with a positive activated protein C/protein C inhibitor complex, there was a good correlation between activated protein C/α_1-antitrypsin and activated protein C/protein C inhibitor complex levels ($r = 0.619$, $p < 0.05$).

To assess whether the difference between the levels of activated protein C/α_1-antitrypsin and activated protein C/protein C inhibitor observed both in normal pregnancy and in pathologic pregnancy is due to changes in the levels of protein C inhibitor or α_1-antitrypsin, the activated protein C serine protease inhibitors were measured in all groups. The functional

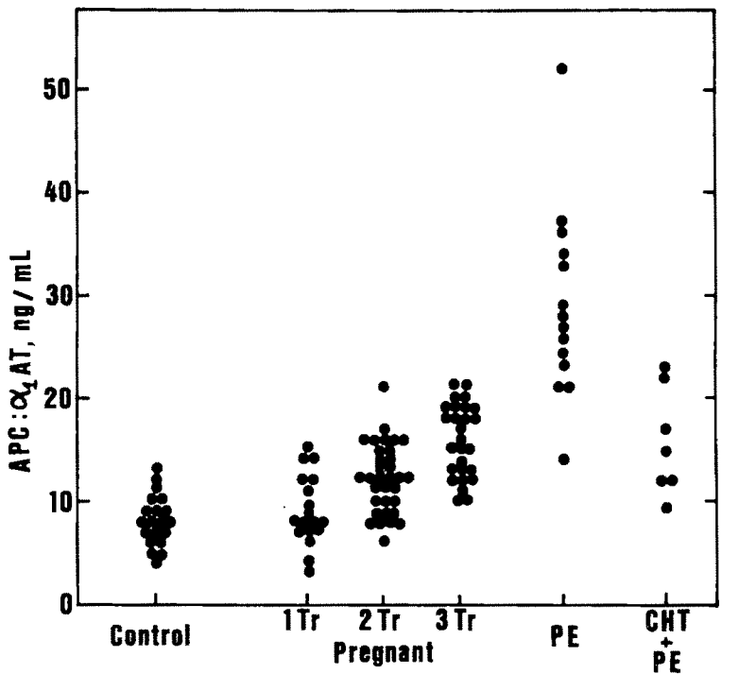


Fig. 1. Activated protein C/α_1-antitrypsin *(APC : α_1AT)* complex levels in control nonpregnant women (n = 20), in women with normal pregnancy during the first *(1 Tr,* n = 18), second *(2 Tr,* n = 33), and third trimesters *(3 Tr,* n = 27), in severe preeclampsia *(PE,* n = 14), and in chronic hypertension with superimposed severe preeclampsia *(CHT + PE,* n = 7). Activated protein C/α_1-antitrypsin complex levels in pregnant women at second and third trimesters were significantly higher ($p < 0.001$) than those of controls. The activated protein C/α_1-antitrypsin complex level in severe preeclampsia, but not in chronic hypertension with superimposed severe preeclampsia, was significantly higher ($p < 0.001$) than that of women in the third trimester.

and antigenic protein C inhibitor levels of pregnant women at different gestational ages were compared with those of nonpregnant controls (Table II). The pregnant women showed a mean functional protein C inhibitor level of 86% ± 15% (mean ± SD) (first trimester), 82% ± 12% (second trimester), and 77% ± 18% (third trimester). The nonpregnant women had a mean functional protein C inhibitor level of 96% ± 21% with a range of 62% to 139%; the difference between these levels and those of the three groups of pregnant women was significant at the $p < 0.05$ (first trimester), $p < 0.01$ (second trimester), and $p < 0.001$ (third trimester) levels. The group with severe preeclampsia showed a significantly decreased mean functional protein C inhibitor level (63% ± 26%, $p < 0.01$) as compared with that of normal pregnant women in the third trimester of pregnancy, whereas the group with chronic hypertension with superimposed severe preeclampsia had an increased level of functional protein C inhibitor (91% ± 16%, $p < 0.05$) as compared with the control group of women in the third trimester of pregnancy. Protein C inhibitor antigen levels were similar to functional protein C inhibitor levels in all groups except in the third trimester of pregnancy, in which the functional protein C inhibitor level was significantly reduced as compared with the antigen protein C inhibitor level ($p < 0.05$).

The values of α_1-antitrypsin in normal and pathologic pregnancy and in the nonpregnant control group are seen in Table II. The nonpregnant women had a mean α_1-antitrypsin level of 96% ± 9% with a range of 78% to 130%. The mean α_1-antitrypsin levels of pregnant women increased during pregnancy and were significantly increased (139% ± 29%, first trimester; 143% ± 16%, second trimester; 158% ± 24%, third trimester) as compared with that of the nonpregnant women at the $p < 0.001$ level. Both the group with severe preeclampsia and the group with chronic hypertension and superimposed severe preeclampsia had similar α_1-antitrypsin levels (164% ± 35% and 156% ± 19%, respectively) as compared with those of the control group of normal pregnant women in the third trimester of pregnancy.

Protein C, both antigen and amidolytic activity, also was measured in all groups (Table II). No significant alterations were found in the level of protein C during

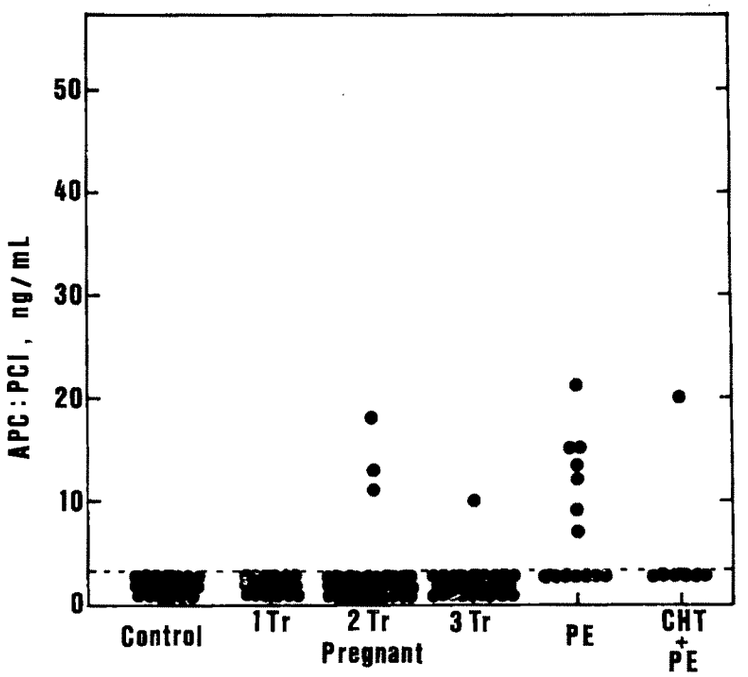


Fig. 2. Activated protein C/protein C inhibitor *(API:PCI)* complex levels in controls and in normal and pathologic pregnancies. Groups are same as in Fig. 1. Difference between complex levels in women with normal pregnancy and women with severe preeclampsia was significant at $p < 0.001$.

Table II. Coagulation parameters in controls (nonpregnant women), in normal pregnancy during first, second, and third trimesters, in patients with severe preeclampsia, and in patients with chronic hypertension and superimposed severe preeclampsia

	No. of patients	*Protein C (%)*		*Protein C inhibitor (%)*		
		Antigen	*Functional*	*Antigen*	*Functional*	*α_1-Antitrypsin (%)*
Controls (nonpregnant)	20	98 ± 16	95 ± 14	98 ± 16	99 ± 18	96 ± 9
Normal pregnancy						
First trimester	18	97 ± 12	95 ± 13	86 ± 16	86 ± 15*	139 ± 29†
Second trimester	33	95 ± 17	94 ± 15	87 ± 11	82 ± 12‡	143 ± 16†
Third trimester	27	93 ± 21	90 ± 19	86 ± 16	77 ± 18†	158 ± 24†
Preeclampsia	14	80 ± 23	75 ± 21*	70 ± 21‡	63 ± 26*	164 ± 35
Chronic hypertension with superimposed severe preeclampsia	7	96 ± 14	91 ± 16	94 ± 18	91 ± 16*	156 ± 19

Values are mean ± SD. Values of normal pregnancy were compared with those of control group and values of patients were compared with those of normal pregnancy at third trimester.

*$p < 0.05$.

†$p < 0.001$.

‡$p < 0.01$.

normal pregnancy, whereas functional protein C was significantly decreased in severe preeclampsia as compared with the levels of the normal pregnant women at a similar gestational age (third trimester). In contrast, patients with chronic hypertension with superimposed severe preeclampsia showed protein C values similar to those of normal pregnant controls.

Comment

Our results show a progresive increase in activated protein C/α_1-antitrypsin complex level during normal pregnancy, reaching values twice that of the control group in the third trimester (Fig. 1), i.e., 16 versus 8 ng/ml. In contrast, only 5% of the pregnant women had detectable levels of activated protein C/protein C

inhibitor complex (Fig. 2). These relative amounts of the two activated protein C complexes seen in pregnancy are similar to those found in patients with thrombotic disease[19] and do not coincide with in vitro[8] and in vivo[9, 19] data suggesting that the two types of activated protein C complexes are formed at approximately the same rate. One possibility is that in vivo, activated protein C/protein C inhibitor is cleared faster than activated protein C/α_1-antitrypsin as indeed is the case in baboons.[19a] Another possibility is the presence of hitherto unrecognized in vivo factors that modulate the relative rates of complex formation.

The group of patients with severe preeclampsia showed a significant increase in the level of activated protein C/α_1-antitrypsin as compared with normal women in the third trimester of pregnancy and with patients with chronic hypertension and superimposed severe preeclampsia, whereas the group of patients with chronic hypertension and superimposed severe preeclampsia had activated protein C/α_1-antitrypsin levels similar to those in the third trimester of pregnancy. This likely reflects basic differences in pathogenic mechanisms between severe preeclampsia and chronic hypertension with superimposed severe preeclampsia.

To ascertain whether the increase in the levels of activated protein C/inhibitor complexes was the result of a variation in the levels of the inhibitors, we measured the protein C inhibitor and α_1-antitrypsin levels in all groups. Although there is a significant increase in the α_1-antitrypsin levels in pregnancy, no correlation between levels of activated protein C/α_1-antitrypsin and those of α_1-antitrypsin was found in any of the groups studied. Moreover, the α_1-antitrypsin levels of patients with severe preeclampsia were similar to those of the group with chronic hypertension with superimposed severe preeclampsia and those of the control group in the third trimester, but the level of activated protein C/α_1-antitrypsin complex in patients with severe preeclampsia was twice that of the two other groups. These data suggest that the increase in the level of activated protein C/α_1-antitrypsin complex during pregnancy and in severe preeclampsia is not due, or at least not principally due, to the increase in the level of α_1-antitrypsin. Rather, it may reflect greater activation of the protein C system that is due in turn to greater thrombin activity. Moreover, activation of the intrinsic coagulation pathway has been reported to occur in preeclampsia.[20] As a result, kallikrein is generated that could interact with protein C inhibitor, resulting in protein C inhibitor inactivation[18, 21] and making α_1-antitrypsin the major inhibitor of activated protein C. In fact, we found significantly decreased levels of protein C inhibitor activity and antigen in preeclampsia (Table II).

In summary, our results support the hypothesis that a hypercoagulable state exists in both normotensive and hypertensive pregnant patients and that the increased levels of thrombin result in an overactivation of the protein C pathway. Evidence for this prethrombotic state in normal pregnancy and in preeclampsia was also provided by Wallmo et al.[22] and Weiner et al.[22] who reported a significant rise in fibrinopeptide A levels in pregnancy[22, 23] and in preeclampsia,[22] and by de Boer et al.[24] who found increased thrombin–antithrombin III levels in normal and hypertensive gestations. Our results also support previous suggestions[18, 19] that the activated protein C/α_1-antitrypsin complex could be a suitable marker for specific detection of activation of the clotting and protein C pathways. Activated protein C/α_1-antitrypsin could also aid in the differential diagnosis of severe preeclampsia versus chronic hypertension with superimposed severe preeclampsia and represents a reasonable initial screening for the coagulation abnormalities accompanying preeclampsia.

We thank Mrs. Josefa Solano for her excellent technical assistance.

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Progress in pathogenesis and management of clinical intraamniotic infection

Ronald S. Gibbs, MD,[a] and Patrick Duff, MD[b]
Denver, Colorado, and Gainesville, Florida

In the past decade, gratifying progress has been achieved in our understanding of clinical intraamniotic infection. With a usual incidence of 1% to 4%, clinical intraamniotic infection mainly develops as an ascending prccess after prolonged rupture of the membranes and labor, but other cases may be hematogenous in origin whereas still others complicate intrauterine procedures. The most common organisms isolated in amniotic fluid of cases of intraamniotic infections are anaerobes, genital mycoplasmas, group B streptococci, and *Escherichia coli.* The latter two are found most commonly in maternal or neonatal bacteremia complicating intraamniotic infection. Although the diagnosis remains largely a clinical one, laboratory tests have been suggested to confirm the diagnosis in women with symptoms. These include amniotic fluid Gram stain, gas-liquid chromatography, and leukocyte esterase measurement. Maternal treatment consists of antibiotic therapy and delivery. Studies to date have used a penicillin plus an aminoglycoside, with some authors advocating the addition of clindamycin after cesarean delivery. Other broad-spectrum regimens may be equally effective. Complications of clinical intraamniotic infections include an increase in cesarean section rate and in maternal and neonatal bacteremia. Poor neonatal outcomes in intraamniotic infection are more likely in the following cases: (1) when *E. coli* or group B streptococci are present in the amniotic fluid; (2) when the infant has a low birth weight; (3) when maternal antibiotic therapy is delayed until after delivery. (Am J Obstet Gynecol 1991;164:1317-26.)

Key words: Clinical intraamniotic infection, prolonged rupture of membranes, antibiotic therapy

Clinical intraamniotic infection is a common, acute, clinically detectable infection primarily of the uterus and its contents during pregnancy. Extragenital manifestations include bacteremia and shock. Several alternative terms are in widespread use and include *clinical chorioamnionitis, amnionitis, amniotic fluid infection,* and *intrapartum infection.* (In this review we use the term given by the original authors.)

Intraamniotic infection is distinct, however, from histologic chorioamnionitis, a condition defined histopathologically by the presence of polymorphonuclear infiltrates in the placenta and its membranes. Histologic chorioamnionitis occurs far more frequently than does clinically evident intraamniotic infection. At term, histologic chorioamnionitis occurs in approximately 20% of pregnancies but is infrequently associated with clinically evident intraamniotic infection. Before term, histologic chorioamnionitis occurs much more frequently, recently being reported as high as 60% in pregnancies with birth weights between 1000 and 1500 gm.[1]

From the Department of Obstetrics and Gynecology, University of Colorado Health Sciences Center,[a] and the Department of Obstetrics and Gynecology, University of Florida College of Medicine.[b]
Reprint requests: Ronald S. Gibbs, MD, Box B198, University of Colorado Health Sciences Center, 4200 E. 9th Ave., Denver, CO 80262.
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Definition of intraamniotic infection

The diagnosis of intraamniotic infection has in general been based on fever, usually with membrane rupture, and a variety of other physical and laboratory findings. For the purposes of their prospective studies, Gibbs et al.[2] defined intraamniotic infection on the basis of temperature ≥100° F (37.8° C) plus two or more of the following conditions: maternal tachycardia, fetal tachycardia, uterine tenderness, foul odor of the amniotic fluid, or maternal leukocytosis. (Because these studies involved exclusively patients who had amniotic fluid samples aspirated by intrauterine pressure catheter, all of the patients also had ruptured membranes.)

Incidence

In the past the overall incidence of this condition was reported as being approximately 1% to 2%. In a ret-

Table I. Prospective studies of risk factors for intraamniotic infection

Feature	*Newton et al.*[5]	*Soper et al.*[6]
Site	San Antonio	Richmond
No. studied	2908	408
Prevalence of intraamniotic infection (%)	4.2	10.5
Independent risk factors by logistic regression analysis	Parity	No. of vaginal examinations
	Duration of ROM	Duration of ROM
	Duration of ROM	Use of IFM
		Duration of labor

ROM, Rupture of membranes; *IFM*, internal fetal monitoring.

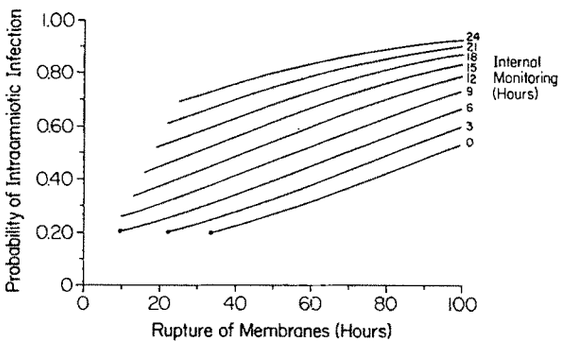


Fig. 1. Probability of intraamniotic infection in nulliparous women as determined by logistic regression. (Reprinted with permission from The American College of Obstetricians and Gynecologists. From Newton ER, Prihoda TJ, Gibbs RS. Logistic regression analysis of risk factors for intra-amniotic infection. Obstet Gynecol 1989;75:571-5.)

rospective study in 1980 Gibbs et al.[3] reported an incidence of 0.8% in a teaching hospital in San Antonio. Koh et al.[4] at Los Angeles County Hospital reported a similar incidence at approximately the same time.

Two recent prospective studies,[5, 6] also at teaching hospitals, reported higher incidences, 4.2% and 10.5%, respectively. It is unclear whether the incidence has truly increased over the past decade, perhaps because of increased use of invasive techniques during labor, or whether the older rates underestimated the true occurrence of the disease.

Pathogenesis

Most cases of intraamniotic infection are ascending in origin, commonly after prolonged rupture of the membranes and labor in patients with multiple examinations. However, occasionally intraamniotic infection may occur in patients with labor but without membrane rupture.

A second route of intraamniotic infection is hematogenous spread in mothers with bacteremia. Most notably, *Listeria monocytogenes*, which may occur either as an epidemic or in isolated cases, has been reported as a cause of blood-borne intraamniotic infection.[7] In addition, there have been cases of hematogenous intraamniotic infection caused by group A streptococci and *Campylobacter* sp.[8]

A third mechanism for the development of intraamniotic infection is introduction of bacteria during an invasive procedure. After diagnostic amniocentesis, the risk of acute intraamniotic infection is low, probably best estimated as 1 in 1000.[9] With more extensive manipulations, such as intrauterine transfusions, the risk of acute infection may be estimated as high as 5%.[10] Intraamniotic infection also may be a complication of cervical cerclage. Most studies indicate that the likelihood of intrauterine infection after placement of a cervical cerclage is in the range of 1% to 2%,[11] but in patients who have a cerclage placed after marked dilatation and prolapse of the membranes into the vagina, the risk of intrauterine infection may be as high as 25%.[12] Percutaneous umbilical blood sampling has occasionally been the cause of fetal loss (1.4%, 36/2501), but approximately half of the losses (n = 17) are due to chorioamnionitis (Berkowitz R. North American Periumbilical Blood Sampling Registry. Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990).

Coitus during pregnancy has been suggested as a risk factor for *amniotic fluid infection*.[13] However, Mills et al.[14] in a study of 10,000 Israeli women demonstrated that frequency of intercourse decreased during pregnancy, from >90% of women in the first 6 months to 36% of women in the ninth month, and that women who had intercourse did not have an increased risk of premature rupture of the membranes, low birth weight, or perinatal death.

In 1989 two prospective, sophisticated studies of risk factors for intraamniotic infection appeared. At the Medical College of Virginia, Soper et al.[6] prospectively evaluated 408 women for chorioamnionitis. Newton et al.[5] in San Antonio evaluated 2908 women. With an overall incidence of 10.5% and 4.2%, respectively, in these two studies, independent risk factors were identified by logistic regression. These are shown in Table I. With their logistic regression model, Newton et al.[5] were able to develop probability curves that are based on parity, duration of internal fetal monitoring, and duration of ruptured membranes, as shown in Fig. 1, which shows a curve that was developed for nulliparous women. In this model the probability of intraamniotic infection was estimated at 20% in patients who had 20 hours of membrane rupture and 3 hours of internal fetal monitoring. However, the risk of intraamniotic

infection rose to an estimated 40% in patients who had 20 hours of membrane rupture and internal fetal monitoring of 12 hours. It was also important to note from this study that most patients entered a high-risk category a number of hours before development of clinically evident intraamniotic infection. This observation suggests that it might be possible to implement intervention strategies to prevent clinically evident intraamniotic infection.

More than 35 years ago, investigators recognized that amniotic fluid may contain substances that inhibit bacterial growth. In the late 1960s and the 1970s a number of groups identified various amniotic fluid antibacterial factors. Most notably, the group at the University of Iowa[15] reported isolation of a low-molecular-weight peptide with a zinc cofactor that inhibited bacterial growth. Subsequently, Blanco et al.[16, 17] reported that amniotic fluid from patients with intraamniotic infection was significantly less likely to inhibit the growth of *Escherichia coli* and group B streptococci.

However, when Silver et al.[18] subsequently attempted to isolate a specific amniotic fluid bacterial inhibitor, they were not able to demonstrate any bacterial inhibitory property of amniotic fluid once they corrected for the major effect of pH and a minor effect of osmolality. At present, it is unclear whether there are specific bacterial inhibitors in amniotic fluid and whether these are clinically relevant.

Microbiology

The microbiologic composition of amniotic fluid in patients with intraamniotic infection is indeed complex and is similar to that of several other obstetric infections. In 1982, Gibbs et al.[19] published a case-control study of the microbiologic features of amniotic fluid in 52 patients who had clinically evident intraamniotic infection and in 52 matched, case controls. Seventy percent of the patients with intraamniotic infection had $\geq 10^2$ CFU/ml of virulent organisms, but in comparison only 8% of patients in the control group had this finding ($p < 0.001$). The most common organisms in patients with intraamniotic infection in this series were anaerobes and group B streptococci. The next year, this group[20] reported that *Mycoplasma hominis* was present in the amniotic fluid of 35% of patients with intraamniotic infection and in only 8% of matched controls ($p < 0.001$), whereas *Ureaplasma urealyticum* was present in 50% of patients in both groups. Most patients who had *M. hominis* in the amniotic fluid also had $>10^2$ CFU/ml of virulent organisms (83%, 15/18). In 1982 Gravett et al.[21] reported similar findings in a smaller series. In 1988 the results of amniotic fluid culture in 408 cases of intraamniotic infection were summarized[22] (Table II).

Recently, interesting interactions of organisms in the amniotic fluid of these cases became apparent. Two constellations of organisms were noted.[23] In one, *Gardnerella vaginalis, M. hominis,* and anaerobes appeared to be associated with each other in the amniotic fluid. When one of these organisms was present in the amniotic fluid, it was significantly more likely that another member of this constellation also would be present. In the second set of interactions between organisms, enterococci, *E. coli,* and other aerobic gram-negative rods were associated. These patterns of organisms in the amniotic fluid suggested that in the former group bacterial vaginosis–associated organisms might be the source, whereas in the latter series gut-associated organisms might be the source.

Table II. Distribution of microbes in 408 cases of intraamniotic infection[22]

Microbe	*No.*	*%*
Group B streptococci	60	15
Enterococci	22	5
E. coli	33	8
G. vaginalis	99	24
Other aerobic gram-neg. rods	21	5
Peptostreptococci	38	9
B. bivius	120	29
Fusobacterium sp.	23	6
B. fragilis	14	3
M. hominis	125	31
U. urealyticum	193	47

When patients with intraamniotic infection were stratified by birth weights of their infants, we found that pregnancies resulting in low-birth—weight infants (<2500 gm) were more likely to have anaerobes in the amniotic fluid.[22] For example, *Bacteroides bivius* was present in 45.9% of pregnancies with low-birth-weight infants and in 27.9% of pregnancies with non-low-birth-weight infants ($p = 0.035$). Similarly, *Fusobacterium* sp. were present in 21.6% of low-birth-weight pregnancies compared with just 3.8% of non-low-birth-weight pregnancies ($p < 0.001$). It was of interest that there was no difference in the isolation rate of group B streptococci, *E. coli,* or other aerobic organisms or of *M. hominis* or *U. urealyticum.* These data may be interpreted to imply that subclinical anaerobic infection may cause preterm labor or preterm premature rupture of the membranes, in the first place, and then are more likely to be isolated in pregnancies with low-birth-weight infants and clinically evident intraamniotic infection.

Because of the multiplicity of organisms found in the amniotic fluid of patients with intraamniotic infection, serologic studies have been performed to help in the understanding of pathogenicity. Using an enzyme-linked immunosorbent assay for detection of maternal serum antibody, Gibbs et al.[24] found that women who have intraamniotic infection with *M. hominis* in the amniotic fluid have a significantly greater antibody re-

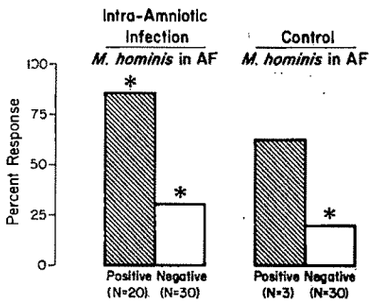


Fig. 2. Antibody response to *M. hominis* (seroconversion and/or rise in A_{400} [absorbance] >0.2) in patients with intraamniotic infection and in control patients. Women with intraamniotic infection and *M. hominis* in amniotic fluid had an antibody response significantly more often than did other groups (*asterisk*, $p < 0.05$) (see ref. 19).

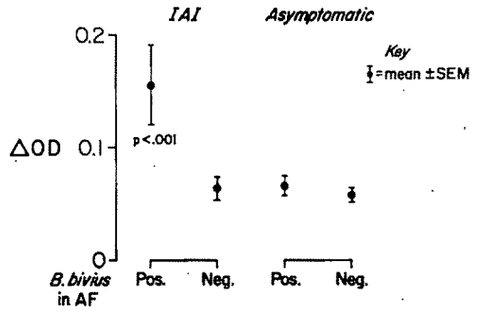


Fig. 3. Antibody response by enzyme-linked immunosorbent assay to *B. bivius* in patients with intraamniotic infection and in control patients. Mean duration of delivery was significantly greater in women with intraamniotic infection and *B. bivius* in amniotic fluid than in any other group (on basis of data in ref. 24).

sponse than do appropriate control subjects (Fig. 2). We observed similar significant antibody changes in patients who had intraamniotic infection and with *B. bivius* in the amniotic fluid (Fig. 3).[25] These serologic studies indicate that these organisms, among others, most likely are pathogenic in intraamniotic infection. On the other hand, no significant antibody changes were identified for either *G. vaginalis* or *Chlamydia trachomatis*.[26, 27]

Even though group B streptococci and *E. coli* are present collectively in only 20% of amniotic fluid samples from women with intraamniotic infection, these two organisms account for 67% of either maternal or neonatal bacteremia.[22, 23] As shown in Table III, when group B streptococci are present in the amniotic fluid from cases of intraamniotic infection, either the mother or the neonate has group B streptococcal sepsis in 25% of cases. With *E. coli* in the amniotic fluid of these cases, bacteremia develops in 33% of maternal-infant pairs. Among 408 cases of intraamniotic infection, organisms causing maternal bacteremia were as follows: group B streptococci ($n = 11$), *E. coli* ($n = 7$), *Staphylococcus aureus* ($n = 2$), *Listeria monocytogenes* ($n = 2$), *Bacteroides* sp. ($n = 2$), and one each with enterococci, viridans streptococci, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*.[23] Bloodstream isolates from newborns in these cases were as follows: group B streptococci ($n = 4$), *E. coli* ($n = 5$), *S. aureus* ($n = 2$), streptococci ($n = 3$), enterococci ($n = 2$), *Enterobacter* sp. ($n = 1$), *Haemophilus influenzae* ($n = 1$), *Haemophilus parainfluenzae* ($n = 1$), and microaerophilic streptococci ($n = 1$).[22] Of note, no cases of anaerobic neonatal bacteremia were documented, and there were only two cases of maternal anaerobic bacteremia. This observation may be interpreted to mean that the anaerobes are active locally but are not invasive organisms. It has also been suggested that current techniques may not be able to detect anaerobic bacteremia, especially with low inoculum size.

Clinical and laboratory diagnosis

Clinical criteria used to make the diagnosis of intraamniotic infection are fever, maternal tachycardia, fetal tachycardia, uterine tenderness, and foul odor of the amniotic fluid, all with or without membrane rupture. Since most patients do not have all of these findings, laboratory tests may assist in making the diagnosis. Of these laboratory measures, maternal peripheral blood leukocyte count is the most readily available. In patients with symptoms several investigators over the past decade have evaluated rapid diagnostic techniques, including amniotic fluid Gram stain (Fig. 4), amniotic fluid gas-liquid chromatography, and amniotic fluid leukocyte esterase.

Maternal leukocytosis. Patients with clinical intraamniotic infection often have a moderate leukocytosis (>12,000/mm³ to 15,000/mm³) with a shift toward more immature cell forms ("left shift"). In a case-control study Gibbs et al.[2] reported leukocytosis (>15,000/mm³) in 63% of cases of intraamniotic infection and in 21% of matched controls. Hoskins et al.[28] found leukocytosis (>12,000/mm³) to be a test with 67% sensitivity, 86% specificity, 82% positive predictive value, and 72% negative predictive value.

Gram stain and culture of amniotic fluid. Gram stain and culture of amniotic fluid are of value in confirming the diagnosis of chorioamnionitis in patients with symptoms,[2] when amniotic fluid usually is obtained through the intrauterine pressure catheter. A positive Gram stain was defined as the presence of any bacteria in a high-power oil-immersion field[2] of uncentrifuged amniotic fluid. White blood cells may be present in amniotic fluid simply as a consequence of labor, and thus they are not necessarily indicative of infection. In this

Table III. Outcomes by organism in amniotic fluid[22, 23]

Organism	*Neonatal sepsis*	*Neonatal death from sepsis*	*Maternal sepsis*	*Maternal or neonatal sepsis*
Total (N = 408)	19 (5%)	4 (1%)	23 (6%)	39 (10%)
Group B streptococci (n = 60)	4 (7%)	2 (3%)	11 (18%)*	15 (25%)*
E. coli (n = 33)	6 (18%)	1 (3%)	5 (15%)*	11 (33%)*
Anaerobes (n = 178)			2 (1%)	2 (1%)

*$p < 0.05$, Compared with overall group.

case-control study organisms were observed in 67% (35/52) of cases of intraamniotic infection versus only 12% (6/52) of controls ($p = 0.001$). Forty-two (81%) cases of intraamniotic infection had detectable leukocytes on direct smear whereas only 15 (29%) control cases had this finding ($p < 0.001$).[2]

Leukocyte esterase assay of amniotic fluid. Polymorphonuclear leukocytes contain several esterase enzymes that are not present in serum, urine, or other body fluids. Recently, a simple chemical test for the presence of one of these enzymes has been developed. The leukocyte esterase reagent strip (Boehringer Mannheim, Indianapolis) identifies the esterase that catalyzes the hydrolysis of an indoxyl carboxylic acid ester to indoxyl. Esterase activity can be measured in the absence of intact cells and is not affected by changes in pH, protein concentration, or bacterial content of the fluid specimen. Hoskins et al.[28] evaluated the leukocyte esterase reagent strip in 21 patients with suspected chorioamnionitis and 21 control subjects without symptoms. Amniotic fluid was aspirated through the intrauterine pressure catheter in all patients. Using the clinical diagnosis of chorioamnionitis as the standard for comparison, the authors found that the leukocyte esterase test showed excellent sensitivity (91%), specificity (95%), and positive and negative predictive values (95% and 91%, respectively).

Gas-liquid chromatography. Gravett et al.[21] were the first to describe the use of gas-liquid chromatography for the diagnosis of chorioamnionitis. They evaluated 16 women with clinical evidence of intrapartum infection and 22 control patients without symptoms. Amniotic fluid was collected from patients by transabdominal amniocentesis, aspiration through the intrauterine pressure catheter, or needle aspiration at the time of cesarean delivery. Gas-liquid chromatography was used to identify organic acids in the amniotic fluid specimens. All amniotic fluid samples contained lactate, which is a recognized byproduct of fetal metabolism. Other organic acids were identified in only one of 22 control specimens, in comparison with 15 of 16 samples from patients with infection ($p < 0.0005$). The authors concluded that acids other than lactate (such as acetate, propionate, and butyrate) were products of bacterial metabolism and that identification of these substances was a highly sensitive and specific test for confirmation of chorioamnionitis.

In spite of the impressive results achieved by Gravett et al., several caveats must be emphasized. First, the instrument that the authors used was equipped with a flame ionization detector that was able to detect minute quantities of organic acids. Not all gas-liquid chromatographs are equally sensitive. Second, such sophisticated instrumentation is not readily available at every hospital, especially during evening and nighttime hours. Third, the test is labor intensive and relatively expensive. Therefore for all of the reasons outlined here gas-liquid chromatography analysis is not a test that can be used routinely for diagnosis of intraamniotic infection. The test appears to have its greatest application in research studies designed to investigate microbiologic and pathophysiologic characteristics.

Histologic examination of placental membranes. Histologic examination of the membranes is of limited value in the anterospective diagnosis of intraamniotic infection. First, in the patient whose infant has not been delivered, biopsy of the chorioamniotic membranes currently is neither practical nor safe. Second, even if the biopsy could be performed safely, the processing of the tissue specimen for histologic examination is relatively expensive and labor intensive. Personnel skilled in histologic interpretation are not likely to be available at all hours of the day and night. Third, the histologic inflammation occurs much more commonly than does the clinical syndrome of chorioamnionitis. In high-risk populations nearly all cases of clinical intraamniotic infection have histologic inflammation in the membranes,[29] yet histologic chorioamnionitis is seen in 11% of all membranes and in >50% of membranes from pregnancies with birth weights <1000 gm,[30] whereas clinical chorioamnionitis is diagnosed much less frequently (usually 0.8% to 4%).[3-5]

Immediate outcome

Older retrospective studies of outcome after intraamniotic infection[3, 4, 31, 32] are summarized in Table IV. In summary, these studies demonstrated that intraam-



Fig. 4. Gram stain of uncentrifuged amniotic fluid from patient with intraamniotic infection, showing numerous gram-positive cocci.

niotic infection was accompanied by a twofold to threefold increase in cesarean section rate. Further, with the mean interval from diagnosis of intraamniotic infection to delivery being 3 to 7 hours, no "critical interval" was noted (i.e., after which maternal or neonatal complications increased).[32] Although perinatal mortality was reported to increase in cases of intraamniotic infection, little of the excess perinatal mortality was directly due to infection and the major contributor to high perinatal mortality was prematurity itself.

Effect on labor. More recent studies have addressed the effect of intraamniotic infection on labor and the effects of birth weight and timing of antibiotics on outcome.

In subsequent studies by Duff et al.[33] and Silver et al.,[34] labor in patients who had clinically evident intraamniotic infection and subclinical infection, respectively, was characterized. Patients with clinically evident intraamniotic infection were most often primigravid with dystocia. Approximately 80% had been receiving oxytocin at the time of diagnosis of intraamniotic infection, and abnormal fetal heart rate patterns were fairly common.[31] Even patients without symptoms but with positive amniotic fluid cultures (suggested by the authors to be "subclinical intraamniotic infection") appeared to require a higher dose of oxytocin to achieve adequate uterine activity.[34] Even with adequate uterine activity, progress in cervical dilatation was slow. Since this abnormal cervical dilatation could not be explained by excessive fetal size, an abnormal pelvis, anesthetics, or other drugs, the authors speculated that infection per se may have some adverse effect on cervical collagen tissue.

Effect of birth weight. Recent controlled studies have demonstrated that intraamniotic infection in pregnancies with low-birth-weight infants is much more likely to result in poor outcome, when compared with non-infected pregnancies at the same gestational age or infected pregnancies at term. In 1982 Garite and Freeman[35] compared pregnancies with and without amnionitis at 28 to 34 weeks' gestation. There was a significant increase in perinatal deaths, respiratory distress syndrome, and total infections in 47 pregnancies with amnionitis as compared with the 204 cases without amnionitis. Ferguson et al.[36] reported a case-control study of perinatal outcome after *chorioamnionitis* in 1985. More than 70% of their cases were in low-birth-weight infants. Although the differences were not statistically significant, the chorioamnionitis cases were accompanied by a higher perinatal mortality rate (20% vs 11%), a higher sepsis rate (6% vs 2%), and a higher asphyxia rate (27% vs 16%). Since all of these adverse effects were higher in the group with chorioamnionitis, we suspect that there may be a substantial β-error in this study in that true adverse effects are not detected. The recent work by Morales,[37] studying 92 patients with chorioamnionitis and 606 control cases of similar gestational ages, also demonstrates significant increases in the group with chorioamnionitis in mortality, respiratory distress syndrome, intraventricular hemorrhage, and clinically diagnosed sepsis (see Table V).

Sperling et al.[22] compared outcome characteristics in cases of intraamniotic infection leading to low-birth-weight infants versus infants of normal weight. The outcome in the former group was characterized by more frequent maternal bacteremia (13.5% vs 4.9%, $p = 0.06$), early onset neonatal sepsis (16.2% vs 4.1%, $p = 0.005$), and neonatal death from sepsis (10.8% vs 0%, $p < 0.001$). Among low-birth-weight infants the interval from rupture of membranes to a diagnosis of intraamniotic infection was short when anaerobes only were isolated in the amniotic fluid compared with when aerobes only were isolated in the amniotic fluid (mean ± SD, 3.8 ± 5.4 vs 39.2 ± 28.3 hours; $p \leq 0.001$). One may interpret these data to show that the significantly shorter interval of low-birth-weight infants with anaerobes in the amniotic fluid suggests that anaerobic intraamniotic infection may have preceded preterm premature rupture of the membranes.

Taken as a whole, these studies clearly demonstrate the adverse effects of intraamniotic infection in pregnancies with low-birth-weight infants.

Selection and timing of antibiotics. In broad principle there is agreement that patients who have acute intraamniotic infection need the general support of therapy, that antibiotic therapy is appropriate, and that delivery is essential to a cure. (There have been a few reported cases of subclinical chorioamnionitis that were cured without delivery.[38] However, in clinically evident disease protraction in delivery may lead to serious, life-threatening maternal infection.) For several decades there was debate as to when antibiotics should be ad-

Table IV. Retrospective studies of outcome after intraamniotic infection

Series	No.	Gestational age (wk, mean)	Cesarean delivery (%)	DDI* (hr)	Perinatal mortality
Koh et al.[4]	140	39	43	—	28/1000
Gibbs et al.[3]	171	36	34	5.2	140/1000
Looff and Hager[31]	104	36	26	7.2	123/1000
Hauth et al.[32]	103	>37	42	3.1	9.7/1000

*Interval from diagnosis of intraamniotic infection to delivery.

ministered. Some favored intrapartum treatment begun promptly to protect the mother from complications such as septicemia and shock. Others argued for delaying treatment until after delivery so that "more thorough cultures of the neonate" might be obtained. Although no agreement exists as to the standard antibiotic selection for intraamniotic infection, most studies to date (both retrospective and prospective) have evaluated a penicillin, usually ampicillin, often in combination with an aminoglycoside. In view of the high failure rate of the ampicillin-aminoglycoside combination in patients with chorioamnionitis *and* cesarean section, we have advocated the empiric addition of clindamycin after cord clamping. Although no studies have been published, it is possible that other regimens may be equally effective. Considering the spectrum of organisms involved in intraamniotic infection, we suspect that several of the newer penicillins, cephalosporins, or penicillin plus β-lactamase inhibitor combinations may be highly successful.

Within the past decade there also has been rising interest among neonatologists in treating early onset neonatal sepsis or meningitis with combination antibiotics other than ampicillin plus gentamicin.[39] These alternative regimens include ampicillin plus a newer, cephalosporin-like antibiotic, such as cefotaxime or ceftriaxone. Similar regimens also may be reasonable in the treatment of intraamniotic infection because they may offer pharmacokinetic advantages over gentamicin and may obviate the need to add clindamycin in patients undergoing cesarean delivery in the presence of intraamniotic infection.

In the past 3 years three studies[40-42] evaluated intrapartum versus postpartum treatment of intraamniotic infection. These three studies consistently demonstrated a lower rate of neonatal sepsis with intrapartum treatment (Table VI).

In 1987 Sperling et al.[40] reported the first systematic study to determine whether maternal intrapartum antibiotic therapy would reduce the incidence of maternal and neonatal complications. In cases collected between 1981 and 1986 they reported a nonrandomized allocation as to timing of treatment (intrapartum versus immediately post partum). All patients were treated with penicillin G (5 million units intravenously every 6 hours) plus gentamicin (1.5 mg/kg every 8 hours). After birth, neonates had blood cultures obtained and chest roentgenography performed within 2 hours of birth, and neonatal treatment with ampicillin plus gentamicin was started for 72 hours in all infants. If neonatal sepsis or pneumonia were diagnosed, the antibiotics were continued for 10 days. During that time interval most patients were treated intrapartum, with postpartum treatment being deferred for those patients who were likely to be delivered within approximately an hour after the diagnosis of intraamniotic infection.

In that study of 257 patients intrapartum therapy was given to 80% ($n = 211$) and postpartum treatment was given to the remainder ($n = 46$). There was no difference in duration of membrane rupture, gestational age, or cesarean section rate, but patients who received intrapartum treatment had a longer mean interval from the diagnosis of intraamniotic infection to delivery (4.7 ± 3 vs 1.8 ± 2.1 hours, $p < 0.001$. Maternal outcome was not different between the two groups, but neonatal sepsis (2.8% vs 19.6%, $p < 0.001$) and neonatal death from sepsis (0.9% vs 4.3%, $p = 0.07$) were decreased in the group receiving intrapartum treatment (Table VI).

Another retrospective study was reported by Gilstrap

Table V. Effect of clinical chorioamnionitis on outcome of preterm infants with premature rupture of membranes[37]

	Chorioamnionitis ($n = 92$)	No chorioamnionitis ($n = 606$)	p Value
Gestational age (wk)	30.2	31.2	
Mortality (%)	25	6	<0.01
Respiratory distress syndrome (%)	62	35	<0.01
Intraventricular hemorrhage (%)	56	22	<0.01
Sepsis (clinical) (%)	28	11	<0.01

Table VI. Effect of timing of maternal antibiotics on confirmed neonatal sepsis*

Series	N	Neonatal sepsis		p Value
		Intrapartum treatment (%)	*Postpartum treatment (%)*	
Sperling et al.[40]	257	2.8	19.6	0.001
Gilstrap et al.[41]	273	1.5	5.7	0.06
Gibbs et al.[42]	45	0	21	0.03

*Positive blood culture.

et al.[41] from Parkland Memorial Hospital in Dallas. One hundred thirty-three cases of chorioamnionitis with intrapartum maternal treatment were compared with 140 cases with no intrapartum treatment (all pregnancies ≥35 weeks). Overall, bacteremia occurred in 1.5% of the intrapartum treatment group versus 5.7% of the group without intrapartum treatment ($p = 0.06$). In particular, group B streptococcal bacteremia occurred in none of the intrapartum treatment group and 5.7% of the group without intrapartum treatment ($p = 0.004$). There were only 39 cases with chorioamnionitis at ≤34 weeks' gestation and no cases of bacteremia were reported. The authors felt that these observations were consistent with the concept that intrapartum treatment decreases neonatal bacteremia.

Because of study design deficiencies of these previous trials and the importance of the clinical question, Gibbs et al.[42] performed a randomized trial of intrapartum versus immediate postpartum treatment in women with intraamniotic infection. By randomization, all mothers received ampicillin (2 gm intravenously every 6 hours) plus gentamicin (1.5 mg/kg every 8 hours). In addition, if cesarean delivery was performed, clindamycin was added post partum. Enrollment in this study was limited to patients with intraamniotic infection at ≥34 weeks' gestation and with cervical dilatation ≥4 cm. We limited enrollment to these patients out of concern for untreated intraamniotic infection with premature infants and for prolonged labor without treatment. (We reasoned that observations in the study group could be applied to the groups specifically excluded.) Vaginal, blood, and amniotic fluid cultures were obtained. After delivery, neonates had blood cultures and a chest x-ray film obtained. A cerebrospinal fluid culture was performed only with referrable signs. Neonatal treatment consisted of ampicillin (75 mg/kg intravenously every 12 hours) plus gentamicin (2.5 mg/kg every 12 hours), begun within 1 to 2 hours after birth. Antibiotics were continued for at least 72 hours. If sepsis or neonatal pneumonia was diagnosed, antibiotics were continued for 10 days. After enrollment of 45 evaluable patients, the study was stopped by joint decision of the investigators and an impartial safety committee.

There were no significant differences between the intrapartum and postpartum treatment groups with regard to any labor parameter or with regard to maternal genital cultures.

Maternal outcome measures consisting of maximum postpartum temperature, postpartum hospital stay, and number of febrile days were all significantly improved in mothers with intrapartum treatment ($p = 0.05$). It is more important, however, that neonatal outcome was improved. With the groups well matched with regard to gestational age and birth weight, early neonatal sepsis occurred in none of the intrapartum treatment group versus 21% of the postpartum treatment group ($p = 0.03$). If we consider sepsis or pneumonia, then these complications occurred in none of the intrapartum treatment group versus 32% of the postpartum treatment group ($p = 0.003$). The decrease in neonatal infections was accompanied by a 2-day shorter hospital stay (3.8 ± 1.1 vs 5.7 ± 3.0, $p = 0.02$). The only late infection (a case of delayed neonatal sepsis) occurred in the postpartum treatment group.

In this study we concluded that intrapartum versus immediate postpartum antibiotic treatment of intraamniotic infection resulted in improved maternal and neonatal outcome.

On the basis of these three studies, the benefits of intrapartum maternal treatment clearly outweigh any theoretic disadvantage such as interference with neonatal cultures.

Route of delivery. As noted, cesarean delivery should be reserved for cases of intraamniotic infection with additional standard obstetric indications for abdominal delivery. The cesarean section rate is increased, however, to approximately 35% to 40% because of its increased likelihood in cases of intraamniotic infection, primarily as a result of dystocia and secondarily fetal distress.[40]

When abdominal delivery is necessary, the standard transperitoneal, low-cervical transverse approach is most commonly used and results in a good outcome. Cesarean hysterectomy should be reserved for patients who have additional obstetric or gynecologic indications for hysterectomy (such as cervical dysplasia or uterine myomas) or supervening obstetric complications (such as refractory uterine atony). Indeed, the likelihood of

uterine atony is increased in patients with intraamniotic infection, as well as in patients who have received high doses of oxytocin during labor. However, in most cases uterine atony may be controlled medically with drugs, including 15-methyl-prostaglandin $F_{2\alpha}$.[43]

In the past several authors[44, 45] have recommended extraperitoneal cesarean section when abdominal delivery was indicated in patients with intraamniotic infection. In the only comparative study, however, Yonekura et al.[46] found no benefit to extraperitoneal cesarean section as compared with transperitoneal cesarean section regarding avoidance of major complications or shortening hospital stay. In addition, in 11.5% of the experitoneal cesarean sections there was inadvertent entry into either the bladder or the peritoneum. Accordingly, extraperitoneal cesarean section, a technique practiced by few obstetricians at any rate, appears to offer no special advantage even in cases of amnionitis.

Long-term outcome

In the last few years, two studies have looked at the influence of clinical chorioamnionitis on long-term prognosis of the infants. In 1985 Hardt et al.[47] compared the outcome of 42 low birth weight infants born from pregnancies complicated by chorioamnionitis to 31 infants of similar weight born after premature rupture of the membranes but without chorioamnionitis. Infants born after chorioamnionitis occurred had significantly lower mental development index scores (104 ± 18 vs 112 ± 14, $p = 0.017$) and lower physical development index scores (92 ± 21 vs 101 ± 21, $p = 0.098$). Two years later, Morales[87] reported these scores in preterm infants born of pregnancies complicated by chorioamnionitis and from control infants born after premature rupture of the membranes but without chorioamnionitis. The mental development and physical development scores were not significantly different. However, cases of chorioamnionitis were matched with controls on the basis of severity of respiratory distress syndrome and intraventricular hemorrhage. Since both respiratory distress syndrome and intraventricular hemorrhage were significantly increased in cases of chorioamnionitis, it appears to us to be inappropriate to match on the basis of these complications. Thus it may be concluded that clinical chorioamnionitis per se may not adversely affect mental and physical development; however, because chorioamnionitis is accompanied by increases in complications such as respiratory distress syndrome and intraventricular hemorrhage, clinical chorioamnionitis may indirectly lead to lower development scores.

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Synergistic effect of intraperitoneally administered calcium channel blockade and recombinant tissue plasminogen activator to prevent adhesion formation in an animal model

Randall C. Dunn, MD,[a] Alex J. Steinleitner, MD,[b] and Hovey Lambert, PhD[b]
Houston, Texas, and Miami Beach, Florida

Previous reports have shown the benefits of calcium channel blockers and recombinant tissue plasminogen activator to prevent postoperative adhesion formation in animal models. To assess the potential benefit of synergistic therapy for the prevention of postoperative adhesion formation, these agents were studied in a rabbit uterine horn model. Four groups of New Zealand White rabbits (n = 8 per group) had a bilateral devascularization injury to the uterine horns. Before closure saline solution, verapamil hydrochloride (2.5 μ/kg/hour), recombinant tissue plasminogen activator (4 mg total dose), or a combination of verapamil and recombinant tissue plasminogen activator at the stated doses were instilled by means of an Alzet osmotic pump × 200 hours. Adhesion scores were evaluated after this time period by estimating the total uterine horn surface involved in adhesions at a terminal laparotomy and by clinically grading the response to determine whether minimal adhesions formed. Results of the total uterine horn surface scores were (mean score ± SE): saline solution, 44% ± 3.7%; verapamil, 19% ± 4.8%; recombinant tissue plasminogen activator, 11% ± 3.6%; combined, 3% ± 1% ($p < 0.01$ to control and $p < 0.05$ to single-drug therapy). Results of the number of animals per group with minimal adhesions were as follows: saline solution, 0; verapamil, 1; recombinant tissue plasminogen activator, 3; combined, 8 ($p < 0.01$). These results show a synergistic benefit of verapamil and recombinant tissue plasminogen activator to prevent postsurgical adhesion formation when delivered via the intraperitoneal route. (Am J Obstet Gynecol 1991;164:1327-30.)

Key words: Calcium channel blocker, recombinant tissue plasminogen activator, adhesions

The development of postoperative adhesions is a significant concern to general and gynecologic surgeons. Their role as a cause of clinical morbidity such as small bowel obstruction or infertility is well recognized. As the pathogenesis of postoperative adhesion formation has become better elucidated, numerous agents have been tested for their ability to reduce the incidence of adhesion formation in the postoperative setting.[1-3] Combinations of different agents to specifically influence certain aspects of the adhesion formation cascade have been attempted with little success.[4-7]

Investigations have shown the individual benefit of calcium channel blockers and recombinant tissue-type plasminogen activator on postoperative adhesion formation.[8-10] Calcium is known to play an important role in the regulation of numerous elements of the inflammatory process and regenerative process.[11] Depressed fibrinolysis predisposes injured peritoneal surfaces to produce early fibrinous adhesions that eventually become collagenous adhesions after the ingrowth of fibroblasts. Decreased plasminogen activator as a result of surgically induced ischemia has been postulated as a unifying pathogenetic mechanism of this process.[12-14]

Because treatment with calcium channel blockade and treatment with recombinant tissue plasminogen activator has each been shown to enhance postsurgical peritoneal repair with less adhesion formation in animal models, this study was undertaken to determine whether the synergistic effects of these two types of agents would enhance their ability to decrease postoperative adhesions compared with the effect of either agent alone.

Material and methods

Twenty-four New Zealand White rabbits weighing 2.75 to 3.25 kg were used as a model for postoperative adhesion formation. Animals were randomly assigned to one of four groups (n = 8 in each group): saline solution control, verapamil hydrochloride (Knoll Pharmaceutical Company, Whippany, N.J.) treatment, recombinant tissue plasminogen activator (Genentech,

From the Department of Obstetrics and Gynecology, Baylor College of Medicine,[a] and the Department of Obstetrics and Gynecology, Mount Sinai Medical Center.[b]
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Reprint requests: Randall C. Dunn, MD, Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX 77030.
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Table I. Incidence of postoperative adhesions after treatment

Group (n = 8)	*Adhesion score* (mean ± SEM)*
Saline solution	44% ± 3.7%
Verapamil	19% ± 4.8%
rt-PA	11% ± 3.6%
Verapamil–rt-PA†	3% ± 1%

rt-PA, Recombinant tissue plasminogen activator.
*$p < 0.01$.
†$p < 0.05$, Compared with single-drug therapy.

Table II. Number of animals with minimal adhesions per group*

Saline solution	*Verapamil*	*rt-PA*	*Verapamil–rt-PA*
0	1	3	8

rt-PA, Recombinant tissue plasminogen activator.
*$p < 0.01$, Between all groups.

Inc., South San Francisco, Calif.) treatment, and both verapamil hydrochloride and recombinant tissue plasminogen activator treatment. Animals were housed and maintained preoperatively, intraoperatively, and postoperatively in accordance with the regulations of the Baylor Small Animal Program.

A low-midline laparotomy was performed under aseptic conditions. A rabbit uterine horn devascularization model was used as an ischemic stimulus for adhesion formation. The uterine horns were devascularized by bipolar cauterization (power setting 40, duration less than 1 second; Malis CMC-1 bipolar electrocautery apparatus, Codman Shurtless Inc., Randolph, Me.) of the uterine mesenteric vascular arcade and its anastomotic branches below the horns so that approximately 3 cm of each horn was rendered ischemic, as documented by uterine horn blanching before closure. Drug delivery was by means of Alzet miniosmotic pumps (model 2001 and 2ML, Alza Corp., Palo Alto, Calif.) sutured on the upper anterior abdominal wall before closure. Drugs and saline solution were reconstituted and installed into the miniosmotic pumps immediately before placement in each animal. In all cases the surgeon was blinded as to which pumps contained experimental drugs or saline solution. The abdominal incisions were closed in two layers with 2-0 and 4-0 polyglycolic acid sutures. Animals were killed on postoperative day 8 and adhesions were scored. Adhesion scores were on the basis of the total uterine horn percentage involvement in adhesions. Adhesion scores were performed in a blinded fashion as to the treatment status of the animal. In addition, adhesions were scored in a clinical fashion: animals with ≤5% of the total uterine horn surface were defined as having minimal adhesions. No additional postoperative adhesion prevention adjuncts were used and no perioperative antibiotics were used.

The drug dosages used were 2.4 μg/kg per hour for verapamil hydrochloride and 4 mg total dose for recombinant tissue plasminogen activator. Delivery rates of the two different miniosmotic pump models achieved total delivery by 200 hours. Dosages were used so that essentially complete delivery of the drugs was accomplished by the end of the study. Doses were used that had previously been effective in the reduction of postoperative adhesions in this or similar models at a level where there was also an opportunity to show synergism.

Statistical analysis was performed by analysis of variance by ranks (Kruskal-Wallis test) among groups. Results are presented as mean ± SE; χ^2 analysis was used to assess the differences in animals with minimal adhesions among groups. Statistical significance was defined as $p < 0.05$.

Results

All 32 animals tolerated the procedure well and were able to be evaluated at the terminal laparotomy. There were no bleeding, infectious, or wound-healing complications clinically observed postoperatively. The uterine horn injury produced adhesions in all control-treated animals, consistent with previous studies with this model. There was no clinical evidence of intraabdominal abscess formation or gangrenous change of any uterine horn at the terminal laparotomy. Adhesions, when they occurred, were primarily between the uterine horns themselves, but also occasionally to the adjacent bowel or bladder.

Results of the adhesion scores are presented in Table I and summarized as follows: saline solution, 44% ± 3.7%; verapamil, 19% ± 4.8%; recombinant tissue plasminogen activator, 11% ± 3.6%; combined verapamil and recombinant tissue plasminogen activator, 3% ± 1% ($p < 0.01$). A significant difference in postoperative adhesion formation was also seen between the two individual drugs' effectiveness and the synergistic effectiveness of the two drugs, $p < 0.05$ (also Table I). Animals with clinically defined minimal adhesions also were statistically different among the groups as presented in Table II. All eight animals in the combined drug group had minimal adhesions, whereas no animals in the saline solution group, one animal in the verapamil group, and three animals in the recombinant tissue plasminogen activator group had minimal adhesions. Without the statistical effect of the combined

drug group, there was no significant difference among the other three groups in the production of minimal adhesions.

Comment

Inasmuch as the mechanisms of postoperative adhesion formation have become understood, multiple modalities to prevent adhesions have been tried, either alone or in combination. The two recent therapeutic modalities presented here, which alone have been shown to enhance normal peritoneal healing, calcium channel blockade by verapamil hydrochloride and increased postoperative fibrinolysis by the addition of recombinant tissue plasminogen activator, have in this study shown increased effectiveness when used synergistically in this animal model.

The mechanism(s) by which calcium channel blockade influences the events of adhesion formation are not certain. Experimental evidence exists for several potential effects on the adhesion formation cascade. Calcium blocking agents are known to modulate the extent of tissue damage after ischemic cellular injury.[15, 16] Calcium channel blockade may also affect other humoral or cellular aspects of the acute inflammatory response. Evidence of its influence on parameters including reduced histamine release, altered prostaglandin release, and platelet aggregation may reduce the inflammatory response to tissue injury.[17-19] Calcium channel blockers' activity have shown a decreased fibrin-rich exudate after the inflammatory response because of fewer microscopic pores in small vessels.[20] After the injury, penetration of fibrin-rich matrices by fibroblasts is inhibited by these agents.[21]

The mechanism by which effects of recombinant tissue plasminogen activator reduced postoperative adhesion formation is a result of enhanced fibrinolytic activity. The importance of the fibrinolytic system in the pathogenesis of adhesions was postulated more than 15 years ago.[12-14] The fibrinolytic system is responsible for clearing the fibrin-rich exudate that is formed after surgical injuries. Plasminogen, the inactive proenzyme, is converted to plasmin by tissue-type plasminogen activator and other plasminogen activators.[22, 23] Tissue-type plasminogen activator's activity is enhanced several hundredfold in the presence of fibrin becaue of lysine binding sites on the fibrin surface; this confines plasmin's main proteolytic activity to the appropriate site. After plasmin has caused fibrin degradation, which results in fibrin-split products, plasmin is released and rapidly inactivated by plasmin inhibitors.[24, 25]

Possible points of synergistic activity in the reduction of postoperative adhesions shown by verapamil and recombinant tissue plasminogen activator were not directly studied in these experiments. From the mechanisms of action of the two drugs already mentioned, a possible mechanism might be explained by a decreased inflammatory response after the initial ischemic injury, leading to a decreased fibrin-rich exudate being released at the surgical site, which in turn is lysed by the enhancing fibrinolytic activity of recombinant tissue plasminogen activator. Multiple factors such as timing of drug intervention and total dose of drug used may further affect the outcome.

In conclusion, a significant synergistic effect on prevention of postsurgical adhesion formation was seen with the use of a calcium channel blocker, verapamil, and recombinant tissue-type plasminogen activator at the stated doses, as compared with either drug alone. Synergistic effects are probably a result of the mechanism of action of these two different drugs and their effects on the postoperative adhesion-formation cascade.

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Endothelin and neuropeptide Y are vasoconstrictors in human uterine blood vessels

Gabriel Fried, MD, PhD,[a, b] and Ulf Samuelson, MD, PhD[a, c]
Stockholm, Sweden

The control of uterine blood flow is regulated by a number of factors including vasoactive substances present in the endothelium and the perivascular nerves. We examined the effects of two vasoactive peptides, endothelin and neuropeptide Y, on segments of human uterine arteries and veins in vitro. We found endothelin, a potent vasoconstrictor in the uterine artery and vein, giving a slow and long-lasting contraction starting at 10^{-9} mol/L. At this concentration, the established vasoconstrictor noradrenaline had almost no effect. Neuropeptide Y had no effect of its own in the human uterine artery, but increased basal tone in uterine veins, and at higher concentrations triggered spontaneous contractions. Neuropeptide Y (10^{-7} mol/L) often, but not always, enhanced the contraction induced by noradrenaline (10^{-6} mol/L). Neuropeptide Y (10^{-7} mol/L) also enhanced the contraction induced by transmural nerve stimulation (20 Hz, 0.4 msec). We conclude that endothelin and neuropeptide Y may modulate the control of human uterine circulation. (Am J Obstet Gynecol 1991;164:1330-6.)

Key words: Endothelin, neuropeptide Y, noradrenaline, neuropeptides, uterine artery

Endothelin is a recently discovered 21–amino acid polypeptide with very strong vasoconstrictor effects that occurs in and is released from vascular endothelial cells.[1, 2] Because of its localization and potency, the peptide has been suggested to be involved in vasospastic disorders and hypertension.[1, 3]

From the Departments of Physiology,[a] Obstetrics and Gynecology,[b] and Plastic and Reconstructive Surgery,[c] Karolinska Institute.
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Reprint requests: Gabriel Fried, MD, PhD, Department of Obstetrics and Gynecology, Karolinska Institutet, S-104 01 Stockholm, Sweden.
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Neuropeptide Y is a 36–amino acid polypeptide originally isolated from porcine brain.[4] With the use of immunohistochemical techniques, neuropeptide Y was found in most peripheral tissues of several species including man. Neuropeptide Y–like immunoreactivity is found together with markers for noradrenaline in a population of peripheral sympathetic neurons, with a particularly rich supply to blood vessels.[5] It is known that noradrenaline causes a concentration-dependent contraction of the uterine artery, mediated primarily by α_1-adrenoreceptors.[6, 7]

In the female reproductive tract noradrenaline and neuropeptide Y nerves have been found around ovarian and uterine vessels as well as in the oviduct and the

Table I. Age, parity, endometrial state at the time of surgery, reason for surgery, and current medication for the 15 patients in the study

Patient No.	*Age (yr)*	*Parity*	*Endometrial state*	*Diagnosis*	*Medication*
1	40	2	Proliferation	Myoma	—
2	38	2	Proliferation	Myoma, menorraghia	—
3	49	1	Proliferation	Myoma, menorraghia	—
4	42	0	Proliferation	Myoma, rapid growth	—
5	38	1	Hyperplasia	Myoma	—
6	37	5	Irregular, proliferation	Menometrorraghia	Diazepam
7	53	2	Irregular, proliferation	Myoma, endometriosis	—
8	40	0	Degenerative	Myoma	Tranexamic acid
9	41	0	Secretion	Myoma	—
10	43	3	Proliferation	Myoma, menorraghia	—
11	49	2	Proliferation	Myoma, menorraghia	—
12	43	3	Secretion	Myoma, menorraghia	Diazepam
13	50	4	Proliferation	Myoma	—
14	59	2	Atrophy	Myoma	—
15	56	3	Atrophy	Myoma	—

myometrium.[8, 9] The myometrial noradrenaline nerves are known to degenerate during pregnancy[10] and it was also found that the coexisting neuropeptide Y disappeared.[8, 11] However, in patients with preeclampsia the myometrial levels of noradrenaline and neuropeptide Y were significantly higher than those in normal pregnant women.[11] The role of endothelin in uterine vessels is unknown. To evaluate the possible significance of noradrenaline and neuropeptide Y nerves and endothelin in the human female reproductive tract, we have examined the effects of neuropeptide Y and endothelin on human uterine vessels in vitro.

Methods

Human uterine and ovarian arteries and veins were obtained from 15 patients aged 46 ± 7 years (mean ± SD, range 37 to 59) undergoing hysterectomy because of myoma or bleeding disorders. No patients with malignant disease or patients receiving radiologic cytotoxic, or antihypertensive therapy were included (see Table I). A total of 36 vessel segments were used. The vessels were excised before, or at most within 10 minutes of, clamping the blood flow, and immediately put in ice-cold Ringer solution. The vessels (outer diameter 2 to 3 mm) were dissected free from connective tissue under a stereo microscope and 2 to 3 mm long circular segments of the vessels were mounted vertically in 5 ml organ baths containing Krebs-Ringer solution (37° C) of the following composition (in millimoles per liter): sodium chloride 120.3, potassium chloride 5.9, calcium chloride 2.5, magnesium chloride 1.2, sodium bicarbonate 15.5, sodium biphosphate 1.2, glucose 11.5. The baths were bubbled with a gas mixture of 94% oxygen and 6% carbon dioxide to keep the pH in the bath at 7.35 to 7.45. Care was taken not to damage the endothelium unintentionally. In some preparations the endothelium was removed by gently rubbing with a forceps. The presence and absence of the endothelium was confirmed by addition of acetylcholine (10^{-7} mol/L) or substance P (10^{-8} mol/L) to preconstricted vessels. In vessels with intact endothelium the substances produced a pronounced relaxation. The vascular tension was recorded isometrically with a Grass FT03C force-displacement transducer and registered on a Grass model 7 polygraph. The organ baths were equipped with two platinum electrodes (10 mm long, 10 mm apart); a Grass S44 stimulator was used for electrical stimulation. The vessels were given an initial passive load of 10 to 15 mN and allowed to equilibrate for at least 60 minutes before the experiments.

Drugs were dissolved in 0.9% sodium chloride and added to the baths in volumes of 5 to 50 μl. New additions after wash were performed when a stable baseline was obtained (after at least 10 minutes). When two drugs were added to the same preparation, the interval was usually 5 minutes. The response to the added substances was expressed in percentage ± SEM of the maximal contraction elicited by noradrenaline (10^{-5} mol/L) (E_{max}) for each vessel to minimize variations in absolute contractile response. Dose-response curves were constructed from steady states. Also, pD_2 (the negative logarithm of the agonist concentration inducing 50% of the E_{max}) was computed. The neuropeptide Y initially used was a gift from Dr. K. Tatemoto, but was later obtained from Cambridge Research Biochemicals (Cambridge, United Kingdom); endothelin was obtained from Peninsula Laboratories Europe Inc. (Merseyside, United Kingdom); acetylcholine and substance P were from Sigma Chemical Co., St. Louis).

Statistical analysis was performed with the Mann-Whitney test, and $p < 0.05$ was considered significant.

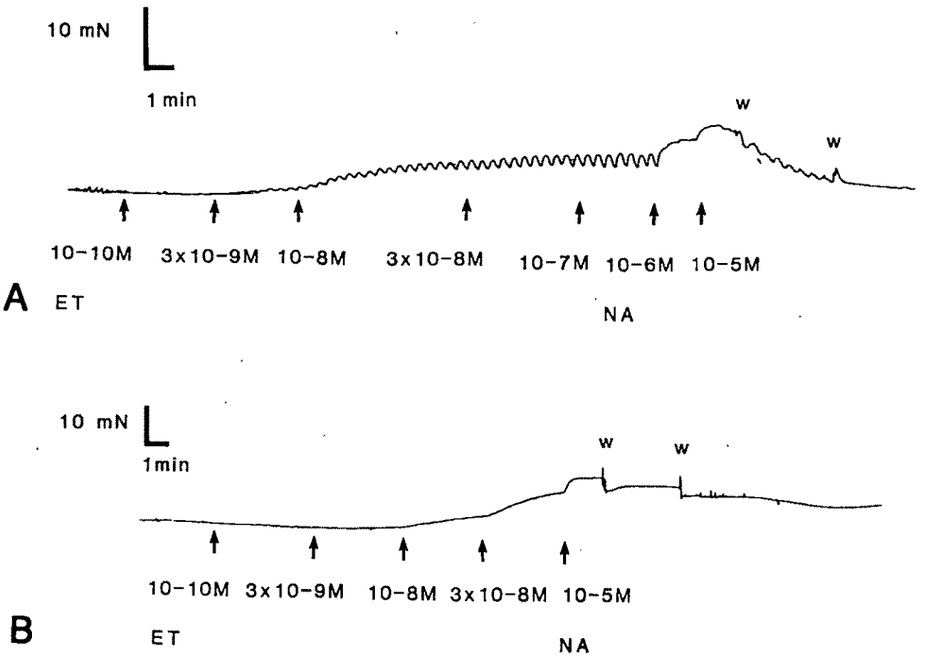


Fig. 1. A, Effect of endothelin (10^{-10} to 10^{-7} mol/L) on human uterine artery. Note slowly developing, long-lasting contraction and gradually developing rhythmic activity. After addition of noradrenaline (10^{-6} to 10^{-5} mol/L) the vessel rapidly contracted further. *w*, Wash. **B,** Effect of endothelin (10^{-10} to 3×10^{-8} mol/L) on human uterine vein. After addition of noradrenaline (10^{-5} mol/L) the vessel contracted further. *w*, Wash.

Table II. Values ± SEM for pD_2 and E_{max} from concentration-response curves to noradrenaline and endothelin

Agonist	pD_2	E_{max}
Noradrenaline	5.9 ± 0.2	95.7 ± 9.0
Endothelin	7.8 ± 0.2	77.0 ± 7.0

pD_2 Value is the negative logarithm of the agonist concentration, causing 50% of the E_{max} response. E_{max} is expressed as the percentage of the maximal stimulation with noradrenaline in the beginning of the experiment.[7]

Results

Noradrenaline at 10^{-8} to 10^{-5} mol/L caused dose-dependent contractions of uterine arteries. The maximal dose caused contractions of 12.0 ± 1.4 mN (n = 39, mean ± SEM). Uterine veins also contracted with noradrenaline, but the contractile force was lower than that in the arteries, 5.4 ± 1.2 mN (n = 9). Phentolamine (10^{-5} mol/L) abolished the contractile response evoked by noradrenaline.

Endothelin induced concentration-dependent contractions of the uterine arteries, starting at 3×10^{-9} mol/L. At 10^{-8} mol/L the contraction was 35.8 ± 7.9% (n = 10), and at 3×10^{-8} mol/L, the contraction was 64.5% ± 13.9% (n = 11) of the maximal noradrenaline contraction. The contraction developed slowly and was always accompanied by a rhythmic activity centered around the increase in tone (Fig. 1, *A*). When NA (3×10^{-6} mol/L) was added during the maximal endothelin-induced contraction, the vessels rapidly contracted further by approximately 30% (Fig. 1, *A*). The vessels relaxed slowly after wash. Uterine veins also responded to endothelin, starting at 10^{-9} mol/L. At 3×10^{-8} mol/L the contraction was 50.0 ± 19.0% of the maximal noradrenaline contraction (n = 4) (Fig. 1, *B*). The concentration-response curves, E_{max} and pD_2 for noradrenaline and endothelin are seen in Fig. 2 and Table II, and show that endothelin is severalfold more potent than noradrenaline on an equimolar basis.

The neuropeptide Y given at doses from 10^{-10} to 10^{-7} mol/L increased basal tone of uterine veins, and at 10^{-7} mol/L often triggered a spontaneous contractile activity (Fig. 3, *B*). The contraction was 36.1 ± 2.6% (n = 7) of the maximal noradrenaline contraction. In uterine arteries there were no clearcut effects on tone or contractility by neuropeptide Y (10^{-10} to 10^{-7} mol/L) given alone.

In uterine arteries, the contraction evoked by noradrenaline (10^{-6} mol/L) was usually enhanced by ad-

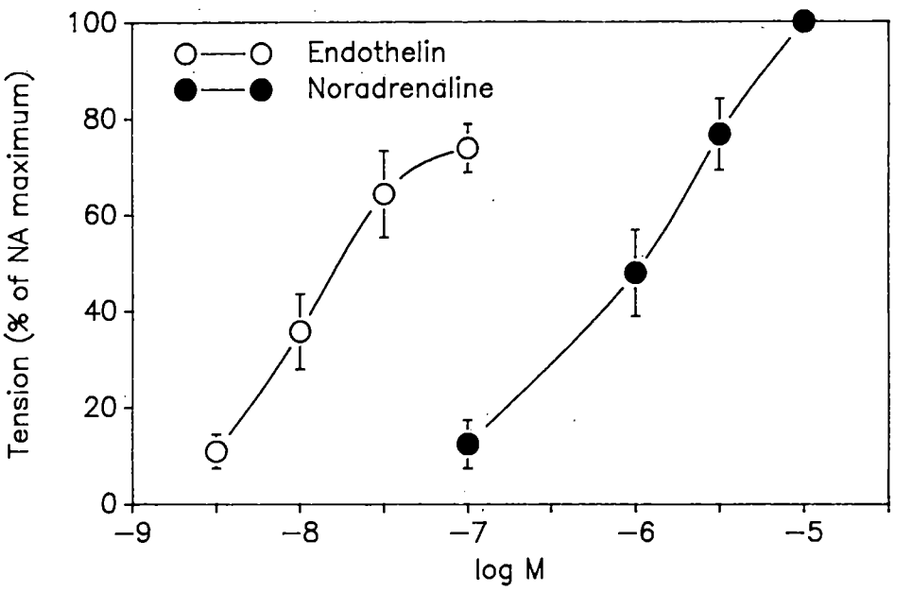


Fig. 2. Concentration-response curve of endothelin and noradrenaline in human uterine artery. On the X-axis, concentration of endothelin or noradrenaline (10^{-9} to 10^{-5} mol/L); on the Y-axis, tension in percentage of maximum tension obtained by noradrenaline (10^{-5}). Each point is the average of three measurements ± SEM.

dition of neuropeptide Y (10^{-7} mol/L) before addition of noradrenaline. The contraction by noradrenaline (10^{-6} mol/L) was 48.0 ± 9.1%, whereas noradrenaline (10^{-6} mol/L) followed by neuropeptide Y (10^{-7} mol/L) increased the contractile response to 76.9 ± 7.4% ($p < 0.05, n = 14$). The increase in the contractile response in this series was at most 60% (Fig. 3, *A*). However, this response was variable, in four vessels an inhibition was seen. The effects of neuropeptide Y appeared to be independent of the endothelium because no difference in reactivity was seen whether the endothelium was rubbed or not. There was no relation between effect of neuropeptide Y and cycle phase or age of the patients. In uterine veins noradrenaline produced a weaker contractile response that was not changed by addition of neuropeptide Y.

In arteries the contractile response evoked by transmural nerve stimulation (20 Hz, 0.4 milliseconds, 60 V, 10-second stimulation with 3-second interval) was slightly potentiated by neuropeptide Y (10^{-7} mol/L) (23.8 ± 4.8% before neuropeptide Y, 40.8 ± 4.2% after neuropeptide Y (10^{-7} mol/L; $p < 0.05$; $n = 5$). The contractile response to field stimulation was not completely blocked by tetrodotoxin (0.1 μg/ml). In uterine veins neuropeptide Y caused an increased basal tone but the amplitude of the stimulation-induced peak was unchanged.

Isolated observations on ovarian vessels gave effects of neuropeptide Y similar to those on uterine vessels. On ovarian veins, neuropeptide Y (10^{-7} mol/L) caused increased basal tone up to 30% to 40% of the maximal and also triggered spontaneous contractions as in uterine veins ($n = 2$). On ovarian artery, neuropeptide Y increased basal tone slightly but did not trigger contractions ($n = 1$).

Comment

These findings show that endothelin is a very potent vasopressor agent in human uterine arteries and veins, and that it is considerably stronger than noradrenaline on an equimolar basis at concentrations below 10^{-7} mol/L. The potent vasoconstrictor effects of endothelin have previously been shown in several vascular beds including the coronary, renal, and skeletal circulation of human beings and the rat.[12-15] In addition, endothelin causes contractions of rat myometrial smooth muscle.[16] The threshold level for activation found in human coronary arteries (10^{-8} mol/L)[13] was similar to that found in the uterine vessels in this study. The potent and long-lasting effects of endothelin can be reversed by dihydropyridine Ca^{++}–channel blockers such as nifedipine and nicardipine,[1, 13, 17] which has led to the suggestion that endothelin may be an endogenous agonist of such Ca^{++} channels.[1, 17] The potent vasoconstriction of the uterine artery elicited by low concentrations of endothelin seen in this study together with

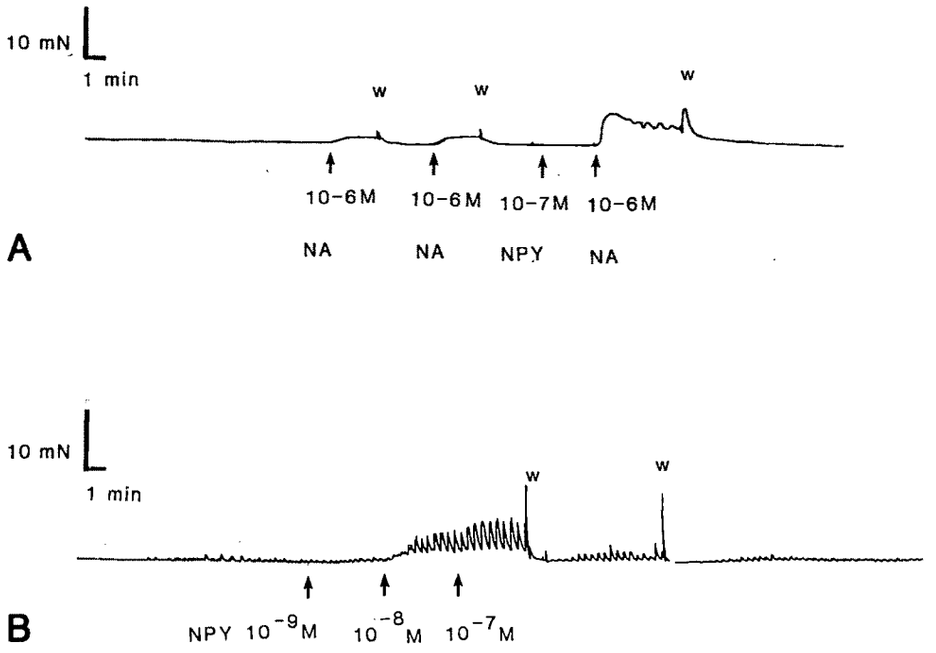


Fig. 3. A, Effects of neuropeptide Y and noradrenaline on human uterine artery in vitro. Neuropeptide Y (10^{-7} mol/L) has no effect by itself, but enhances the contraction caused by noradrenaline (10^{-6} mol/L). *w*, Wash. **B,** Effects of neuropeptide Y on human uterine vein in vitro. Neuropeptide Y causes an increase in tone at 10^{-9} mol/L and triggers spontaneous contractions at 10^{-8} mol/L, which increases amplitude at 10^{-7} mol/L. The effect is reversible and disappears after wash *(w)*.

observations that endothelin is present in vascular endothelial cells indicate that this substance may be involved in vasospastic disorders related to the uterine circulation. However, further investigation of the presence in and release of endothelin from uterine vessels in physiological and pathophysiological states is needed.

The present results also demonstrate that although neuropeptide Y has been reported to be a potent vasopressor in vivo, the effects of neuropeptide Y alone on human uterine vessels in vitro are relatively modest. We found no direct effect on arteries and a moderate vasoconstrictor effect on veins. The ability of neuropeptide Y per se to cause vasoconstriction has previously been demonstrated in various vessels, and the response varied both with vessel type and species. Vessels that have been reported to exhibit a strong sensitivity to neuropeptide Y per se are cerebral arteries and iliac and femoral veins from rabbit and guinea pig.[18-20] It is interesting that small intracervical branches of the human uterine artery appear to be directly sensitive to neuropeptide Y,[21] indicating that there might be regional differences in the reactivity to neuropeptide Y in the uterine vasculature, possibly related to vessel size.[14] Direct effects of neuropeptide Y on the uterine artery have also been seen in the guinea pig, where neuropeptide Y causes long-lasting contractions of isolated arterial segments.[22] Low concentrations of neuropeptide Y (10^{-8} to 3×10^{-7} mol/L) were more potent than equimolar concentrations of noradrenaline, something that has been observed also in vivo on the pregnant guinea pig artery.[23] Other workers have reported only a weak direct effect of neuropeptide Y in the guinea pig uterine artery.[24]

Although we did not see any direct effect of neuropeptide Y on tone or contractility of the uterine artery or ovarian artery, there was a clear effect on uterine and ovarian veins, with increased tone and a triggering of spontaneous contractions. This effect has also been noted in femoral veins.[20]

The potentiating effect of neuropeptide Y (10^{-7} mol/L) on noradrenaline–induced (10^{-6} mol/L) contractions seen in this study has previously been reported both in uterine artery and in other types of arteries.[19, 24, 25] However, not all preparations show potentiation, and the inconsistent response has been examined by Hanko et al.[26] in a large series of experiments on several types of vessels. The reason(s) for this variability is not clear, but does not seem to be related to hormonal status, age of the patients, or endothelial fac-

tors. It may be because of other factors related to the heterogeneous patient material and variations in sample handling. It might also involve interactions with endogenous compounds on the second-messenger level. Support for this view is the observation that neuropeptide Y also potentiates the response to other substances, such as histamine.[19] It was previously determined that interactions between many vasoactive agents, such as noradrenaline and 5-hydroxytryptamine, often cause an enhanced contractile response,[27] indicating that interactions of this kind are not primarily restricted to first-messenger mechanisms.

Transmural nerve stimulation–induced contractions were potentiated by neuropeptide Y in this study, in agreement with several other studies on various vessels in human beings and experimental animals (femoral artery and vein, superior mesenteric artery, portal vein, basilar artery, gastroepiploic artery).[14, 26] Neuropeptide Y also causes a depression of transmural nerve stimulation–evoked tritium-noradrenaline efflux in these vessels and in other organs, such as the fallopian tube and vas deferens.[9, 28] The mechanisms by which neuropeptide Y causes a postsynaptic potentiation of the contractile response in parallell with a presynaptic depression of noradrenaline outflow are not clear but have been discussed in terms of neuropeptide Y–induced changes in muscle tone and concomitantly an increased sensitivity to other vasoactive agents.[14, 26]

The postjunctional contractile adrenergic receptors in the human uterine artery have been pharmacologically characterized in detail as to be primarily of the α_1-type,[6] although α_2-receptors have also been reported.[7] Neither the effects of endothelin or neuropeptide Y are sensitive to α-adrenoreceptor blocking agents, and these two substances appear to mediate their effects through receptors of their own.[16, 20]

In conclusion, this study has shown effects of neuropeptide Y on nonpregnant human uterine artery, indicating that neuropeptide Y modulates vasoconstriction induced by noradrenaline or transmural nerve stimulation. The effects of endothelin on the human uterine artery showed that endothelin induces a slowly developing and long-lasting vasoconstriction. It is suggested that endothelin and neuropeptide Y may modulate the control of human uterine circulation and may be involved in regional vasospastic disorders.

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Behavioral changes in fetal sheep caused by vibroacoustic stimulation: The effects of cochlear ablation

Michael J. Parkes, DPhil,[a] Peter J. Moore, PhD,[a] David R. Moore, PhD,[b] Nicholas M. Fisk, MB, BS,[a] and Mark A. Hanson, DPhil[a]

Reading, England

We measured the effects of 2 minutes of vibroacoustic stimulation on the activity of unanesthetized fetal sheep in utero. We were unable to detect any changes in activity after stimulation with a model 5C electrolarynx. With more powerful stimulation using a mechanical oscillator, fetal electrocortical activity desynchronized. Fetal breathing and eye movements changed with electrocortical activity and there was an increase in nuchal muscle activity. Stimulation caused a small decrease in fetal heart rate. Responses to stimulation were not seen in four of the five fetuses with bilateral cochlear ablation. These results indicate that in fetal sheep the auditory apparatus is necessary for the detection of vibroacoustic stimuli. The difference in responsiveness of the sheep and human fetus appears to be because of a difference in central processing after detection of the stimulus. (Am J Obstet Gynecol 1991;164:1336-43.)

Key words: Fetal sheep, vibroacoustic stimulation, electrocortical activity, heart rate, cochlear ablation

The human fetus in the late phase of gestation is responsive to externally applied vibroacoustic stimulation. A 5-second stimulus with an electrolarynx held against the maternal abdomen causes large and sustained alterations in fetal visceral and somatic motor activity.[1, 2] These include increased heart rate, body movements, and eye blinking[3] and decreased fetal breathing movements. Because little is known about the central nervous system mechanisms that detect and process vibroacoustic stimuli, we wished to investigate the effects of external vibroacoustic stimulation on chronically instrumented and unanesthetized fetal sheep.

Previous studies have shown that in fetal sheep auditory and somatosensory pathways appear to be functional.[4, 5] Measurements of the passage of externally applied vibroacoustic stimuli into the amniotic cavity indicate that the attenuation can be relatively small, especially of frequencies below 5 kHz, and is similar in

From the Fetal and Neonatal Research Group, Department of Physiology and Biochemistry, University of Reading[a] and the University Laboratory of Physiology, University of Oxford.[b]
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Reprint requests: Michael J. Parkes, DPhil, Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill St., London, England NW3 2PF.
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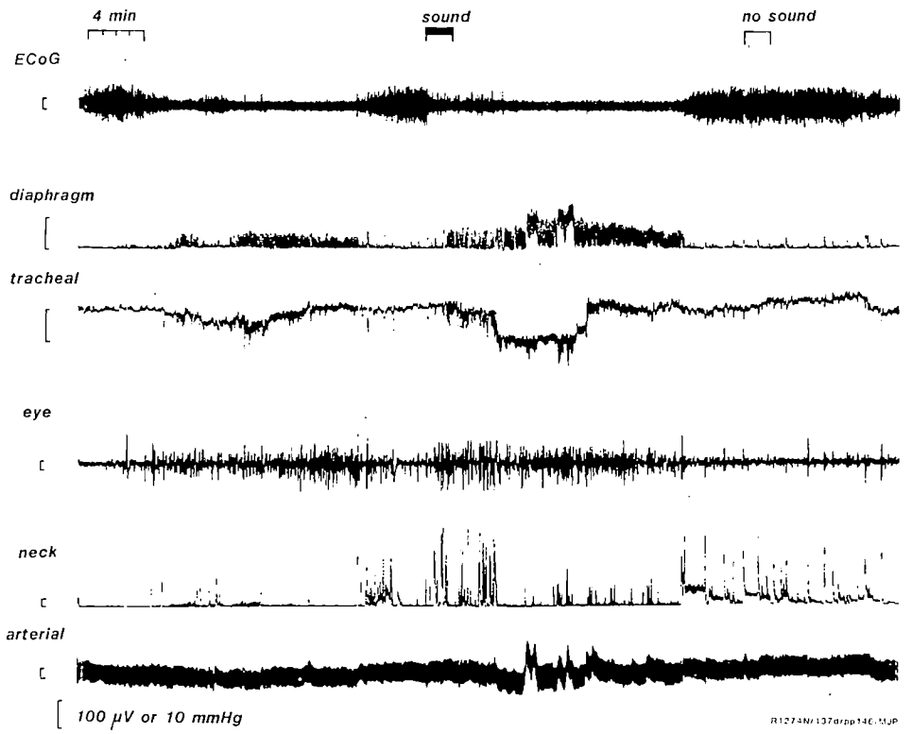


Fig. 1. The effects of stimulation in high voltage electrocortical activity on electrocortical state, diaphragm, eye and nuchal electromyograms, tracheal and arterial minus amniotic pressures in one fetus at 137 days' gestation. *ECoG,* Electrocortical activity. *min,* Minutes.

sheep and man.[6] Fetal sheep also appear to detect external vibroacoustic stimulation, inasmuch as stimulation for up to 45 minutes with a model 5C electrolarynx can alter carbon 14 deoxyglucose uptake in the central nervous system.[7] The behavioral responses of fetal sheep to vibroacoustic stimuli are less clear. Preliminary studies with a number of different stimuli[7-11] indicate that fetal sheep may respond, but stimulation with an electrolarynx[7] was found to produce no significant effect on fetal electrocortical state or any obvious increase in fetal body activity.

Therefore we performed controlled trials in fetal sheep to measure any changes in heart rate and somatic motor and electrocortical activity caused by vibroacoustic stimulation. To measure whether the peripheral auditory apparatus is involved in the detection of external vibroacoustic stimuli, we measured the extent to which these changes were present in fetuses with bilateral cochlear ablation.

Methods

Chronically instrumented fetal sheep were prepared as described previously[12] with standard procedures approved by the host institution and in accordance with United Kingdom legislation. Briefly, 12 cross-bred ewes were anesthetized with halothane (1.5% to 2% in oxygen) at 117 to 124 days' gestation. Electrodes were implanted in the fetus to measure diaphragm and nuchal muscle electromyograms and electroocular and electrocortical activity. Catheters for measurement of pressure were placed in a fetal carotid artery, the trachea, and the amniotic cavity. Catheters and electrodes were brought out through the right flank. In five fetuses cochlear ablation was performed bilaterally with an operating microscope by rupture of the round window, aspiration of the cochlear duct via a 16-gauge needle through the round window, and destruction of the modiolus with round cutting burs. At least 5 days were allowed for postoperative recovery, during which time antibiotics were administered to ewe and fetus. Experiments were performed between 129 to 141 days' gestation when fetuses showed normal episodic breathing and electrocortical patterns and had normal pH and blood gas values (mean pH 7.32, SE 0.01; Po_2 22, SE 0.8 mm Hg; Po_2 45, SE 1 mm Hg). Polygraph records were made at a speed of 5 mm per minute and blood pressure readings were taken at 2 mm per second. Electromyogram signals were filtered at 30 Hz and passed through a leaky integrator.

Source of stimulation. In early trials the stimulus used was 5 to 30 seconds from an electrolarynx (model 5C, Western Electric, New York, New York; measured

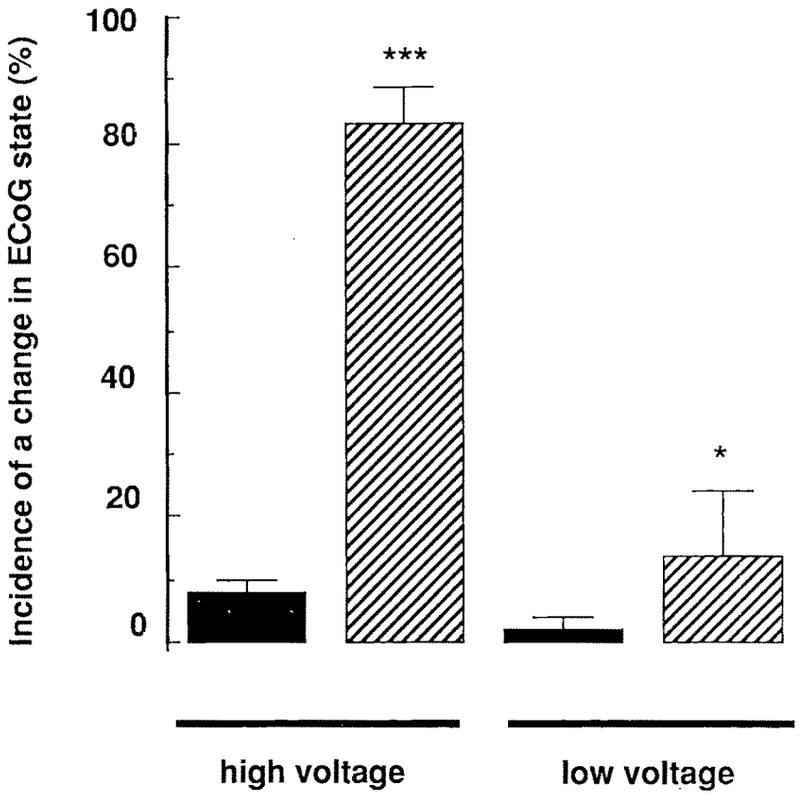


Fig. 2. Mean (±SE) percentage incidence of change in electrocortical state during trials with stimulation *(crossed bars)* or no stimulation *(filled bars)* for trials beginning in high voltage electrocortical state (five fetuses) or low voltage electrocortical state (five fetuses) (n = 252 trials; *one asterisk*, $p < 0.05$; *three asterisks*, $p < 0.001$ for stimulation vs no stimulation in high voltage electrocortical or low voltage electrocortical states). *ECoG*, Electrocortical.

power consumption 0.36 W, disc diameter 3 cm). When this proved ineffective, a function generator was used to provide a square-wave output, the fundamental frequency of which was continuously varied manually between 0.4 and 10 kHz. This stimulus lasted 2 minutes and was applied in every trial. After amplification it was fed into a mechanical oscillator (vibrator 101, Ling Dynamic Systems, Royston, Herts, England) with a measured power consumption from 2 W at 40 Hz to 20 W at 10 kHz. The piston of the oscillator was connected to a plastic disc (diameter 5 cm) covered with ultrasound jelly (Aquasonic 100, Parker Laboratories Inc., Orange, N.J.). Because after surgery the fetal head could not be located with certainty and because the catheters held the fetus to their point of exit (the right side), the piston was applied in every experiment against the right side of the maternal abdomen, approximately 10 cm lateral to the midline. Before experimental trials began, each ewe was accustomed to transducer placement and to the stimulus.

Experimental protocol. Fetal electrocortical state was scored as high voltage or low voltage. When the high voltage electrocortical state was identified, the pretrial period began with the oscillator held against the abdomen of the ewe. After 2 minutes and if still in high voltage, a random number generator determined whether the 2-minute trial was of stimulation or no stimulation. The oscillator was then removed until the next high voltage electrocortical state was identified. The same protocol was followed in the low voltage electrocortical state.

Analysis. During the 2-minute pretrial and trial periods, fetal heart rate (FHR) was counted from the arterial pulse in 10-second intervals; measurements of electrocortical amplitude were made at 36-, 72-, and 108-second points into these periods and also in spontaneous low voltage. During trials the incidence of a change in electrocortical state, the presence of fetal breathing movements, eye movements, and nuchal activity, and the latency (time interval between stimulus onset and first detectable change in electrocortical state) and duration of desynchronizations were measured. On any one day trials were performed in only one electrocortical state, with an average of three trials

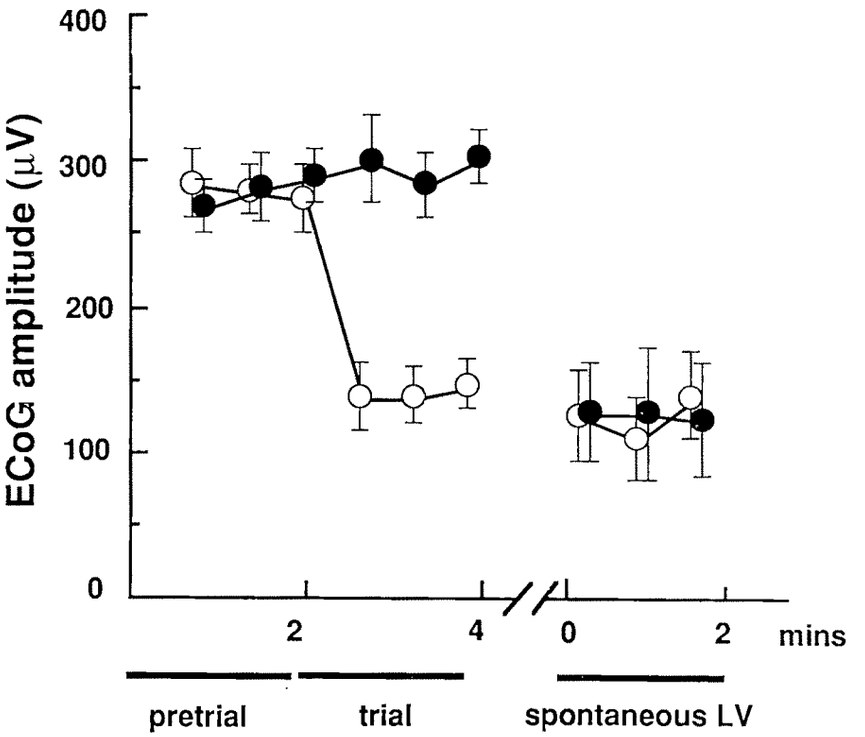


Fig. 3. Mean (± SE) electrocortical amplitude in the 2-minute pretrial and trial periods for stimulation trials begun in high voltage electrocortical state when desynchronization occurred *(open circles)*, no stimulation trials begun in high voltage electrocortical state *(filled circles)*, and in random 2-minute periods of spontaneous low voltage electrocortical state preceding each trial (n = 119 trials in four fetuses). The mean amplitude in the stimulation trial period (131 SE = 5 μV) is significantly lower (*three asterisks*, $p < 0.001$) than that in both pretrial periods (268 SE = 5 μV) and is significantly higher (*one asterisk*, $p < 0.05$) than that in spontaneous low voltage electrocortical state (111 SE = 5 μV). *ECoG*, Electrocortical; *mins*, minutes.

every 2 hours and a maximum of 19 trials. Statistical analysis was performed with standard techniques of nonorthogonal analysis of variance and logistic regression.[13, 14]

Histology. At the conclusion of experiments, animals were killed with pentobarbital (4 gm administered intravenously). Fetuses were perfused via the left ventricle with 10% formalin in saline solution. Cochleae were decalcified by immersion in ethylenediaminetetraacetic acid (EDTA) for 5 months, embedded in paraffin, sectioned every 19 μm, stained with hematoxylin and eosin, and examined under a light microscope.

Results

Intact fetuses. In pilot studies we were unable to detect any changes in fetal activity with 5- to 30-second stimulation with a model 5C electrolarynx in either the high voltage or low voltage electrocortical state, and at any position on the maternal abdomen. The effects of stimulation with the electrolarynx were not investigated further and instead stimuli were applied with the more powerful oscillator. The clearest effect of stimulation with this oscillator was to change fetal electrocortical state and the change was principally from high voltage to low voltage (i.e., a desynchronization). Fig. 1 shows a typical polygraph record and Fig. 2 shows the incidence of a change in electrocortical state in all trials.

In trials begun in the high voltage electrocortical state the incidence of desynchronization after stimulation (84%) was much greater than that occurring spontaneously (8%) (Fig. 2). Every animal responded in at least 69% of stimulation trials. We found no evidence of habituation of this response. Thus the incidence of desynchronization was no different throughout each day and animals continued to respond over several weeks of testing. When desynchronization occurred the mean electrocortical amplitude (131 μV) during the trial was much lower than that in the preceding 2 minutes of high voltage electrocortical state (268 μV), but slightly higher (by 18%; i.e., 20 μV) than that in the spontaneous low voltage electrocortical state (Fig. 3). The latency of the stimulated low voltage electrocortical

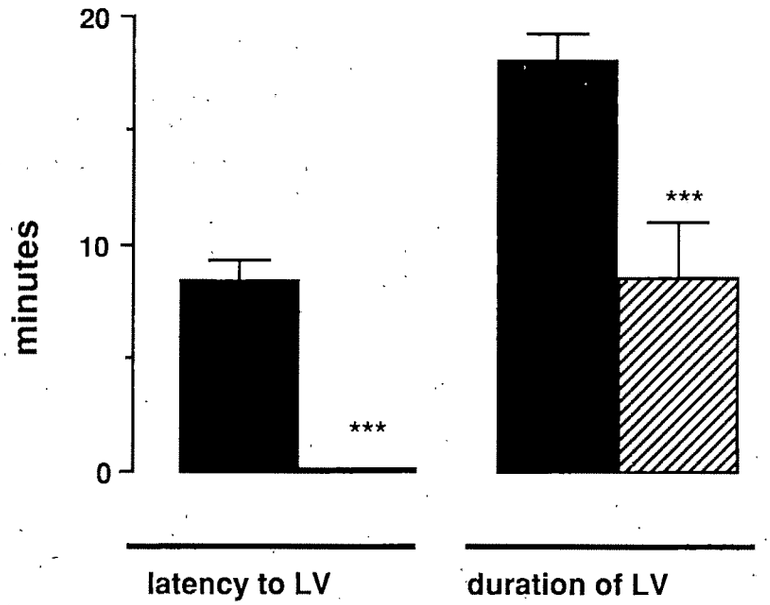


Fig. 4. Mean (±SE) latency and duration of stimulated desynchronization *(crossed bars)* or spontaneous desynchronization when it eventually occurred in no stimulation trials *(filled bars)* for trials begun in high voltage electrocortical state (n = 119 trials in four fetuses). *three asterisks*, $p < 0.001$ for stimulated vs spontaneous desynchronization. *LV*, Low voltage.

state (Fig. 4) was negligible and the mean duration of the stimulated low voltage electrocortical state (Fig. 4) was only half that of spontaneous low voltage electrocortical states.

When stimulation in high voltage electrocortical states caused desynchronization, an episode of fetal breathing and eye movement also occurred. Normally the incidence of fetal breathing movements and eye movements is greatest in low voltage electrocortical states. In four fetuses the mean incidences of fetal breathing and eye movements during induced low voltage electrocortical states (mean 0.57, SE 0.13; mean 0.45, SE 0.25) were not different from those during spontaneous low voltage electrocortical states (mean 0.49, SE 0.07; mean 0.45, SE 0.25). The incidence of nuchal activity was significantly greater during induced low voltage electrocortical states (0.78, SE 0.09) than in spontaneous low voltage electrocortical states (0.21; SE 0.06; $p < 0.01$) or spontaneous high voltage electrocortical states (0.56; SE 0.15; $p < 0.01$). Stimulation in high voltage electrocortical states produced a small but significant fall in FHR (Fig. 5) and had no effect on fetal blood pressure.

In trials begun in the low voltage electrocortical state (Fig. 2) the incidence of changes from low voltage to high voltage electrocortical states (14%) was greater than that occurring spontaneously (2%), but the effect was much less than that in trials begun in the high voltage electrocortical state. Stimulation in the low voltage electrocortical state produced no measurable effects on visceral or somatic motor activity.

Cochlear ablated fetuses. Bilateral cochlear ablations were attempted in five fetuses. After postoperative recovery the incidence, amplitude, and cyclic nature of the spontaneous electrocortical state and of spontaneous motor activity was no different from that in intact fetuses. Fig. 6 shows the incidence of desynchronization during trials in cochlear ablated fetuses. Inasmuch as the variances are dissimilar, statistical analysis by parametric methods is not appropriate, and the results from each animal are presented separately. For comparisons Fig. 6 also shows the results from similar trials in five intact fetuses (taken from Fig. 2 and presented in the same manner). In four of the five cochlear ablated fetuses, the incidence of desynchronization after stimulation was no greater than that occurring spontaneously. Stimulation also did not change visceral or somatic motor activity. Even use of the same stimulus protocol but with lower fundamental frequencies (40 to 400 Hz) and stimulation at maximum intensity did not change the electrocortical state or motor activity. In the remaining fetus, the incidence of desynchronization after stimulation was greater than that occurring spontaneously, but remained less than that in any intact fetus.

Inspection of the cochlear sections under a light microscope revealed varying degrees of damage among the five fetuses. In two fetuses there was gross damage

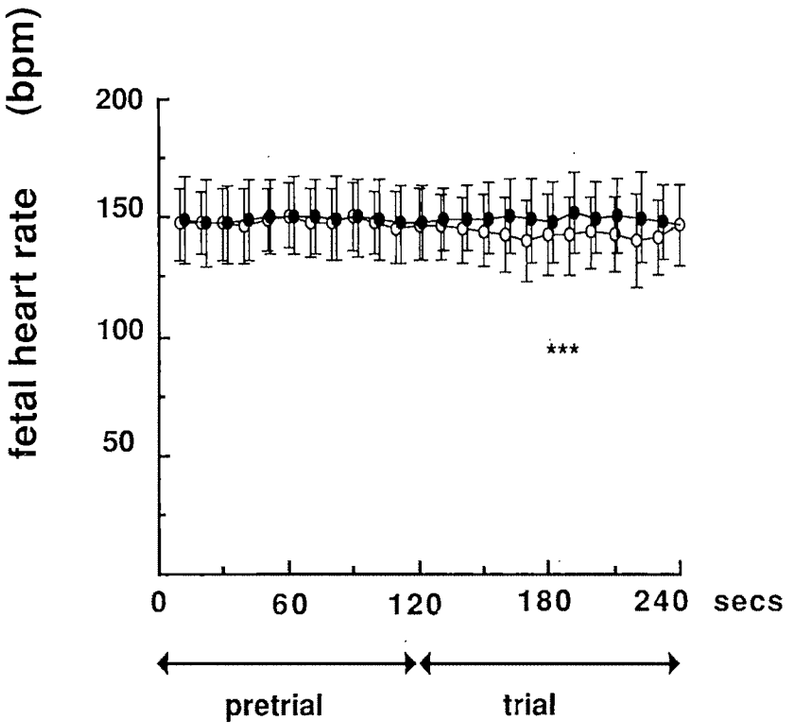


Fig. 5. Mean (±SE) FHR (beats per minute every 10 seconds) in the pretrial and trial periods with stimulation *(open circles)* or no stimulation *(filled circles)* in trials begun in high voltage electrocortical state (n = 63 trials in three fetuses). The overall mean in the stimulation trial period (145 beats/min) is significantly lower (*three asterisks*, $p < 0.001$) than that in either the stimulation pretrial period (149 beats/min) or in the nonstimulation periods (both 150 beats/min). *sec*, Seconds.

to all cochleae in one or more turns. Both these fetuses did not respond to vibroacoustic stimulation. The remaining three fetuses had partial damage in all cochleae in one or more turns and these three could not be distinguished solely on the basis of cochlear damage. Two of these fetuses did not respond to stimulation, whereas in a third the incidence was greater than that occurring spontaneously, but still less than that in any intact fetus.

Comment

We were able to change electrocortical state by stimulation with a powerful oscillator, the most consistent effect being electrocortical desynchronization. The intensity of vibroacoustic stimuli from a point source on the maternal abdomen may vary at different points inside the amniotic cavity. This might influence the incidence of fetal responses, especially if the stimulus is detected by sensory apparatus located in the head. It appears unlikely that consistent movements of the fetal head away from the stimulus influenced the incidence of responses because fetal responsiveness did not decline during each day or over several weeks of testing. It is possible that the fetus with the lowest incidence of responses (69%) was always further away from the stimulus than was the highest responder (100%). Nevertheless, the lowest incidence is still so much greater than the maximum incidence of responses by chance (12%) that a disadvantageous position would clearly not prevent a fetus from responding to this stimulus.

Our pilot studies are consistent with earlier findings[7] that 45-minute stimulation of fetal sheep with the model 5C electrolarynx does not alter fetal electrocortical state. Nevertheless, with the use of intrauterine hydrophones the sound intensity produced by the electrolarynx (135 dB) is considerably higher than that of background noise (55 to 65 dB) in utero.[6] Because 45 minutes of stimulation with the electrolarynx increased glucose uptake in the fetal central nervous system,[7] it is likely that this stimulus is detected by the peripheral sensory apparatus. However, because 45 minutes of such stimulation failed to change the electrocortical state or motor activity,[7] it is likely that the afferent input is of relatively low intensity. We do not know the intensity of the stimulus produced by our oscillator in the amniotic fluid. However, the intensity of the afferent input it produces is likely to be relatively greater than that produced by the electrolarynx. This is because our

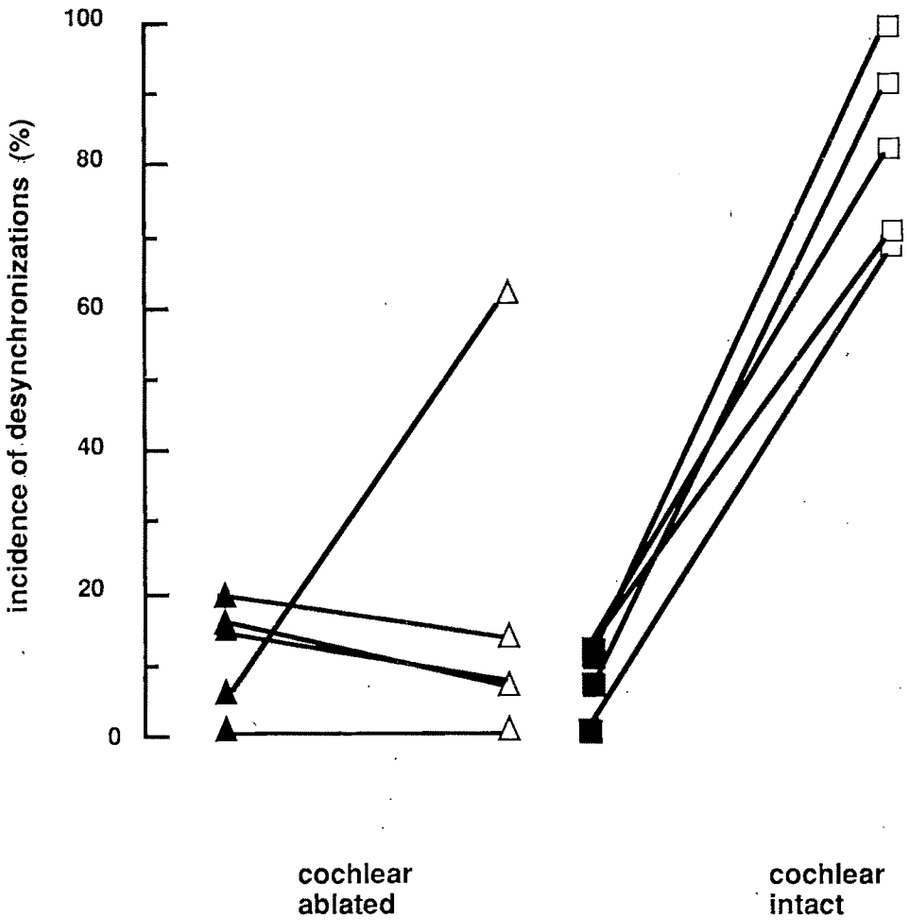


Fig. 6. The incidence of desynchronization in stimulation *(open characters)* and no stimulation trials *(filled characters)* begun in high voltage electrocortical state in five cochlear ablated fetuses (n = 120 trials), and five intact fetuses (n = 155 trials, replotted from Fig. 2).

oscillator was 55 times more powerful and because our oscillator did produce changes in fetal behavior, unlike the electrolarynx.[7] Furthermore, the responses to stimulation with our oscillator are very similar to the responses made by unanesthetized fetal sheep when given direct and intense electric stimulation in utero. Application[15] of 50-volt stimuli for 5 minutes through electrodes chronically implanted into fetal brachial nerve produced almost identical effects on electrocortical state and nuchal muscle activity, with no consistent change in FHR or blood pressure.

The induced low voltage electrocortical state was accompanied by a normal incidence of fetal breathing and eye movements. Its duration and amplitude, however, were 50% shorter and 18% higher than those of the spontaneous low voltage state, and it was accompanied by an abnormally high incidence of nuchal activity. With the use of Fourier analysis on fetal sheep, two transitional electrocortical patterns have recently been described mathematically[16] in addition to the two obvious high voltage and low voltage electrocortical states that are accompanied by characteristic fetal-breathing and eye-movement patterns. It is possible that our induced low voltage electrocortical state contains relatively more of such transitional activity. It is not known whether the spontaneous low voltage state in utero is a unique intrauterine state or is the same as one of the postnatal states of wakefulness or rapid eye movement sleep. Definitions of wakefulness usually require demonstration of purposeful activity,[8] which are not easily made in utero, and sleep needs to be distinguished from a comatose state. Wakefulness and two states of sleep have been suggested to occur in fetal sheep delivered into a warm bath with intact umbilical cords.[8] In that study, however, it was not shown whether any of those three states were the same as the high

voltage and low voltage states observed in utero. For these reasons we have not given a title to the low voltage state induced by vibroacoustic or electrical stimulation.

In fetal sheep after bilateral cochlear ablation, a large reduction in basal glucose uptake in brainstem auditory nuclei has been described.[17] Whereas spontaneous fetal behavior appeared normal, smaller reductions in basal glucose uptake were also observed in many other areas of the fetal central nervous system. It was speculated that cochlear ablation might result in diffuse inactivation of the fetal central nervous system. In all our fetuses with bilateral cochlear ablation we can confirm that spontaneous electrocortical state and visceral and somatic motor activity were normal. Thus the proposed loss of central nervous system activation from auditory structures produces no obvious changes in spontaneous fetal activity. In four of five fetuses with cochlear ablation vibroacoustic stimulation failed to produce the changes in electrocortical state or motor activity that were seen in intact fetuses. In two of these four fetuses there was gross damage in all cochleae and in two there was partial damage. The fifth fetus responded more than expected by chance but less than any intact fetus. There was no obvious difference between the partial cochlear damage in this fetus and that in the two that did not respond, so we are unable to define precisely the damaged cochlear structures responsible for this reduced response. These results nevertheless indicate that in fetal sheep the peripheral auditory apparatus is necessary for the detection of vibroacoustic stimuli.

The initial aim of these experiments was to test whether vibroacoustic stimulation of fetal sheep could produce the same effects as those previously described in human fetuses. In man, the electrolarynx produces a mean increase of 11 beats/min in FHR, with a change apparent up to 1 hour afterward.[1] In sheep, the fact that the electrolarynx increases glucose uptake in the central nervous system indicates that the stimulus is detected, but stimulation with the electrolarynx appears to produce no effect on FHR or behavior. Both our more intense auditory stimulus and direct electric stimulation of the brachial nerve used by others[15] caused a change in electrocortical state with concomitant changes in somatic motor activity, although the overall effect on FHR was negligible. This negligible effect on heart rate cannot be explained by a complete lack of sensory input to cardiovascular efferent centers in the sheep because sudden elevation of fetal blood pressure will induce a reflex fall in FHR.[18] Thus the difference in heart rate response between the two species appears to have its origin in the central nervous system after detection of the stimulus.

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Magnesium sulfate–induced relaxation of uterine arteries from pregnant and nonpregnant patients

Sharon H. Nelson, PhD, and Maya S. Suresh, MD
Galveston, Texas

The effects of magnesium sulfate at different concentrations were investigated in isolated rings of uterine arteries from pregnant and nonpregnant patients. Addition of magnesium sulfate (0.5 to 9.6 mmol/L) to the suffusion medium containing the normal concentration of 1.2 mmol/L magnesium sulfate produced concentration-dependent relaxation of norepinephrine (1 μmol/L)–induced or potassium chloride (35 mmol/L)–induced contractions. Magnesium sulfate was about three times more potent in causing inhibition of potassium chloride–induced contractions in uterine arteries from pregnant patients than in those from nonpregnant patients. The concentrations that inhibited 50% of the contraction induced by potassium chloride were 0.6 ± 0.2 mmol/L (n = 4) in the arteries from pregnant patients and 1.6 ± 0.2 mmol/L (n = 4) in the arteries from nonpregnant patients. At high concentrations (4.8 and 9.6 mmol/L), magnesium sulfate also was more potent in inhibiting norepinephrine-induced contractions in arteries from pregnant women than in arteries from nonpregnant women. The magnesium sulfate–induced relaxation was almost completely inhibited by calcium chloride (2 mmol/L) added to the suffusion medium containing the normal concentration of 2.5 mmol/L calcium chloride but was not affected by indomethacin (5 μmol/L), methylene blue (10 μmol/L), or removal of the endothelium. The results show that magnesium sulfate, at therapeutic blood concentrations, acts as a potent dilator of human uterine arteries, especially those from pregnant patients. The results are consistent with the view that magnesium sulfate may facilitate uteroplacental perfusion. (Am J Obstet Gynecol 1991;164:1344-50.)

Key words: Vascular smooth muscle, norepinephrine-induced contraction, potassium chloride–induced contraction, calcium, endothelium-independent vasodilation

Magnesium sulfate, when it is used parenterally as an adjunct in the management of certain hypertensive disorders during pregnancy, has been reported to produce a slight increase in uteroplacental blood flow in pregnant sheep[1] and to cause an increase in uterine artery blood flow secondary to a selective decrease in uterine artery vascular resistance in the pregnant monkey.[2] Although resistance to flow is usually thought to involve small arteries and arterioles, uterine vascular resistance in pregnant patients may occur in the larger uterine arteries (uterine and radial), because the myometrial resistance vessels (spiral arteries and arterioles) lose the smooth muscle layer early in pregnancy.[3] One objective of the present study was to determine if pregnancy alters the dilator potency of magnesium sulfate in the uterine artery.

The vasodilator activity of magnesium sulfate, which is well documented in a number of other blood vessel preparations,[4] is generally thought to be due to interference with excitation-contraction coupling by competition with calcium.[4-7] However, other mechanisms of action for magnesium sulfate–induced vasodilation have been considered, e.g., the release or action of vasodilator substances such as endothelium-derived relaxing factor[7, 8] or prostacyclin.[4, 9, 10] A second objective of this study was to determine whether magnesium sulfate–induced dilatation of the isolated uterine arteries involves a calcium antagonist action or the release of an endogenous relaxing factor. Preliminary reports of these studies have been published previously.

From the Department of Anesthesiology, The University of Texas Medical Branch.
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Reprint requests: Dr. Sharon H. Nelson, Depart. of Anesthesiology, The University of Texas Medical Branch, Galveston, TX 77550.
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Material and methods

Specimens of the ascending branch of the uterine artery were obtained from patients undergoing hysterectomy for various medical reasons. (Use of uterine arteries from patients undergoing hysterectomy was approved by the Institutional Review Board for the University of Texas Medical Branch, Galveston.) Nonpregnant patients (n = 9) varied in age from 31 to 46 years and were in different phases of the menstrual cycle. Immediately postpartum (pregnant) patients (n = 5) varied in age from 23 to 37 years. The gestations were term.

The nonpregnant patients generally received diazepam or morphine sulfate and atropine, scopolamine, or glycopyrrolate for preanesthetic medication. The pregnant patients received Bicitra Willen antacid for aspiration prophylaxis. Anesthesia was induced with thiopental sodium and maintained with nitrous oxide–oxygen and either halothane, enflurane, or isoflurane.

Immediately after hysterectomy, sections of the uterus containing the uterine arteries were placed in gassed (95% oxygen and 5% carbon dioxide) Krebs-bicarbonate solution and taken to the laboratory. The preparation of isolated arterial rings and the suffusion techniques used have been described in detail previously.[11, 12] In brief, rings (2 mm in length) were prepared from the uterine artery and mounted between two tungsten wires in a 5 ml volume suffusion chamber. Changes in isometric tension were measured by means of a force-displacement transducer (Statham) connected to the upper mounting wire and recorded on a Gould recorder (2400 Brush Model).

After the arteries were mounted in the chambers, they were suffused continuously with gassed (95% oxygen and 5% carbon dioxide) Krebs-bicarbonate solution at a flow rate of 4 ml/min delivered by a peristaltic pump (Gilson Instruments). The Krebs-bicarbonate solution had the following composition: (sodium chloride, 119 mmol/L; sodium bicarbonate, 25 mmol/L; potassium chloride, 3.6 mmol/L; magnesium sulfate, 1.2 mmol/L; potassium phosphate, 1.2 mmol/L; calcium chloride, 2.5 mmol/L; glucose, 11 mmol/L; ascorbic acid, 0.005 mmol/L; and disodium ethylenediaminetetraacetate, 0.03 mmol/L. The bath solution was maintained at 37° C and had a pH of 7.4. The drugs were administered in the inflowing suffusion fluid.

The preparation was allowed a stabilization period of 1 to 2 hours before the experiment began. During that time, basal tension was adjusted repeatedly until the resting tension became stable at 1 gm. (One gram has been found to be the optimal resting tension for human uterine arteries.) After this stabilization period, the contractile responses to norepinephrine at various concentrations were reproducible for several hours.

To determine the effect of magnesium sulfate on resting tension, magnesium sulfate (4.8 mmol/L) was added for 15 minutes to the arterial ring suffused with Krebs-bicarbonate solution containing the normal concentration of magnesium sulfate (1.2 mmol/L). The concentration of 4.8 mmol/L was selected because it produced near-maximal relaxation of precontracted arteries. At least 60 minutes was allowed to wash out the elevated concentration of magnesium sulfate before other studies were done in the same arterial ring.

Contractile responses induced by norepinephrine and potassium chloride were studied in rings from the same artery. The uterine arteries in Krebs solution containing the normal concentration of magnesium sulfate (1.2 mmol/L) were contracted by norepinephrine (1 μmol/L) or by potassium chloride (35 mmol/L). These concentrations were found in previous studies to elicit about 50% to 60% of the maximal contractile responses to these drugs in uterine arteries from pregnant or nonpregnant patients.[12] In the studies with potassium chloride, phentolamine (1 μmol/L) was present in the Krebs solution to antagonize the action of any norepinephrine released from the sympathetic nerve endings by potassium chloride. When the contractile response to norepinephrine or potassium chloride was stable (after 10 to 15 minutes), various experimental protocols were used to examine the effects and mechanism of action of magnesium sulfate.

Magnesium sulfate, at increasing concentrations from 0.5 to 9.6 mmol/L, was added to the contracted artery. Each concentration of magnesium sulfate was administered from 10 to 15 minutes or until the contraction reached a steady state. Throughout these studies magnesium sulfate was administered in Krebs-bicarbonate solution that contained the normal magnesium sulfate concentration of 1.2 mmol/L.

Magnesium sulfate (2.0 mmol/L) was added for 15 minutes to the contracted artery. The concentration of 2 mmol/L was selected because it produced about 50% to 70% inhibition of the contractions induced by norepinephrine. Magnesium sulfate was then washed out until the contractile response to norepinephrine was regained; subsequently, norepinephrine was washed out until the baseline tension returned. This procedure was termed the magnesium sulfate–relaxation protocol. The tissue was then suffused for 20 minutes with a given concentration of indomethacin, to inhibit prostaglandin synthetase, or methylene blue, to prevent the effect of endothelium-derived relaxing factor on soluble guanylate cyclase. Norepinephrine, in the presence of the blocker, was again administered; the magnesium sulfate–relaxation protocol followed. Only one concentration of magnesium sulfate was used in these studies because of the length of time the experiment required.

In studies on the dependency on an intact endothelium for the vasodilatory effect of magnesium sulfate, the magnesium sulfate–relaxation protocol was followed. Subsequently, the endothelium was disrupted mechanically,[13] and a stabilization period of about 30 minutes was allowed before the magnesium sulfate–relaxation protocol was repeated. Successful removal of endothelial cells from the rings was confirmed by histologic examination of the intimal surface with a silver-staining technique.[13] Uterine arteries that had not been through the endothelium removal procedure were also examined by this technique. Control exper-

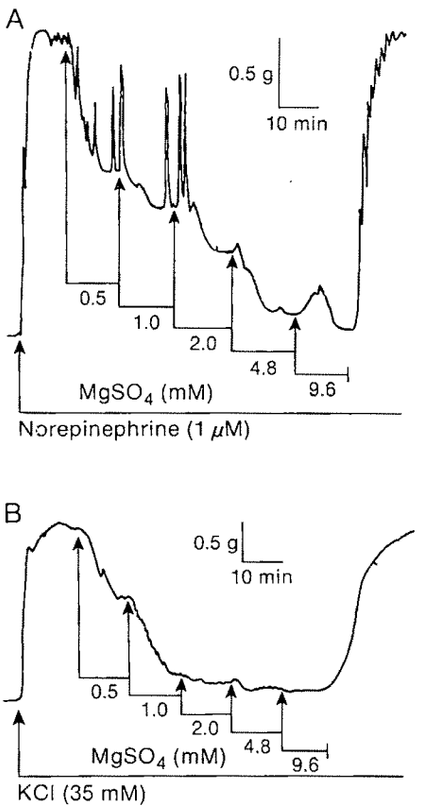


Fig. 1. Inhibition by magnesium sulfate *(MgSO4)* of norepinephrine-induced **(A)** and potassium chloride *(KCl)*–induced **(B)** contractions of isolated uterine artery from a pregnant patient. Concentrations of magnesium sulfate are those added to Krebs solution containing 1.2 mmol/L magnesium sulfate. Each experiment was carried out on a different arterial ring from the same uterine artery. Note that the vasorelaxant effect of magnesium sulfate was readily washed out and that it was more potent when inhibiting a potassium chloride–induced contraction. Endothelium was present in both rings.

iments were performed using the magnesium sulfate–relaxation protocol without the presence of antagonist or blocker or without the disruption of the endothelium.

To investigate a magnesium-calcium interaction in the uterine artery, the magnesium sulfate–relaxation protocol was followed for the control response to magnesium sulfate. The tissue was then suffused for 20 minutes with Krebs solution before norepinephrine was again administered. After the contractile response to norepinephrine stabilized, the concentration of calcium chloride in the Krebs solution was increased to 4.5 mmol/L and allowed to suffuse for 10 to 15 minutes. Magnesium sulfate (2 mmol/L) was then added for 15 minutes in the presence of the elevated concentration of calcium chloride.

Inhibition by magnesium sulfate of the norepinephrine-induced or potassium chloride–induced contraction was expressed as a percentage of the contraction before magnesium sulfate, at various concentrations, was administered. Norepinephrine-induced tonic contraction was often associated with phasic contractions (drug-induced oscillatory activity). When this oscillatory activity occurred, the lowest developed tension during the rhythmic activity was taken as the response; i.e., the amplitude of the rhythmic activities was not included in the measurements. Data are presented as mean values ± SE for grams of contraction, percent inhibition by various concentrations of magnesium sulfate, and the concentrations that inhibited the norepinephrine-induced or potassium chloride–induced contraction by 50%. The values were obtained from graphs of data from each experiment. Results were analyzed by means of a nonparametric analysis of variance test (Wilcoxon). Data obtained with a given protocol in uterine arteries from nonpregnant patients were compared with data obtained by the same protocol in uterine arteries from pregnant patients. A p value <0.05 was considered significant.

All stock solutions and serial dilutions made in Krebs-bicarbonate solution were prepared shortly before the start of each of the experiments. The following drugs were used and were obtained from Sigma Chemical Co.: (-)-arterenol bitartrate, indomethacin, and methylene blue.

Results

The mean arterial blood pressure of the nonpregnant patients was 95.3 ± 5.6 mm Hg (range, 83 to 110 mm Hg; $n = 9$). The mean arterial blood pressure of the pregnant patients was 91.3 ± 3.0 mm Hg (range, 87 to 97 mm Hg; $n = 5$).

After the uterine arteries were mounted in the perfusion chamber and the resting tension was applied, tension gradually increased for 5 to 15 minutes, then decreased. The decrease in tension was often associated with some spontaneous contractile activity. After the baseline stabilized, there was, as a general rule, little or no spontaneous contractile activity. In the uterine arteries from either pregnant patients ($n = 3$) or nonpregnant patients ($n = 3$), addition of magnesium sulfate (4.8 mmol/L) had no demonstrable effect on the resting tension.

In order to examine the relaxing effect of magnesium sulfate in uterine arteries, tone was first induced by either norepinephrine (1 μmol/L) or potassium chloride (35 mmol/L). Developed tension induced by norepinephrine (1 μmol/L) was not significantly different in arteries from pregnant patients (2.4 ± 0.2 gm, $n = 5$) and those from nonpregnant patients (2.7 ± 0.3 gm, $n = 9$), nor was there any significant difference in the magnitude of contraction produced

by potassium chloride (35 mmol/L) in arteries from pregnant patients (3.5 ± 2.1 gm, $n = 4$) and nonpregnant patients (3.2 ± 1.1 gm, $n = 4$). Magnesium sulfate (0.5 to 9.6 mmol/L), added to Krebs solution containing the normal concentration of 1.2 mmol/L magnesium sulfate, produced concentration-dependent relaxation of contractions induced either by norepinephrine or by potassium chloride in arteries from both pregnant (Fig. 1) and nonpregnant patients. Besides inhibiting the norepinephrine-induced tonic contraction in the uterine arteries, magnesium sulfate also inhibited the norepinephrine-induced phasic contractions. The results from experiments similar to those shown in Fig. 1 are summarized in Fig. 2. Magnesium sulfate at low concentrations (0.5 to 2 mmol/L) produced similar inhibition of the norepinephrine-induced contractions in arteries from pregnant and nonpregnant patients (Fig. 2, *A*). The concentrations that inhibited norepinephrine-induced contractions by 50% were 1.1 ± 0.3 mmol/L ($n = 5$) and 0.8 ± 0.4 mmol/L ($n = 9$) in arteries from pregnant and nonpregnant patients, respectively. However, at higher concentrations (4.8 to 9.6 mmol/L) magnesium sulfate produced a significantly greater inhibition of the norepinephrine-induced contraction in arteries from pregnant patients than in those from nonpregnant patients. Magnesium sulfate (4.8 mmol/L) produced nearly complete relaxation of the norepinephrine-induced contraction in the arteries from pregnant patients. In contrast, magnesium sulfate, at all concentrations used in these studies, produced a significantly greater inhibition of potassium chloride–induced contraction in the arteries from pregnant patients than in those from nonpregnant patients. (Fig. 2, *B*). The concentration of magnesium sulfate needed to inhibit potassium chloride–induced contractions by 50% in arteries from pregnant patients (0.6 ± 0.2 mmol/L, $n = 4$) was less than half that required in arteries from nonpregnant patients (1.6 ± 0.2 mmol/L, $n = 4$).

Calcium chloride (2 mmol/L) had little or no effect on the contractile response to norepinephrine but prevented magnesium sulfate (2 mmol/L)–induced inhibition of norepinephrine-induced contraction (Figs. 3 and 4). Similarly, calcium chloride (2 mmol/L) antagonized magnesium sulfate (2 mmol/L)–induced relaxations of potassium chloride (35 mmol/L)–induced contractions in arteries from both pregnant and nonpregnant patients. Magnesium sulfate–induced relaxations of potassium chloride–induced contractions in arteries from pregnant and nonpregnant patients were, respectively, 92% ± 2% ($n = 4$) and 70% ± 8% ($n = 4$) in the absence of calcium chloride (2 mmol/L) and 9% ± 5% ($n = 4$) and 4% ± 7% ($n = 4$) in the presence of calcium chloride. After a 30-minute washout of the elevated concentration of calcium chloride,

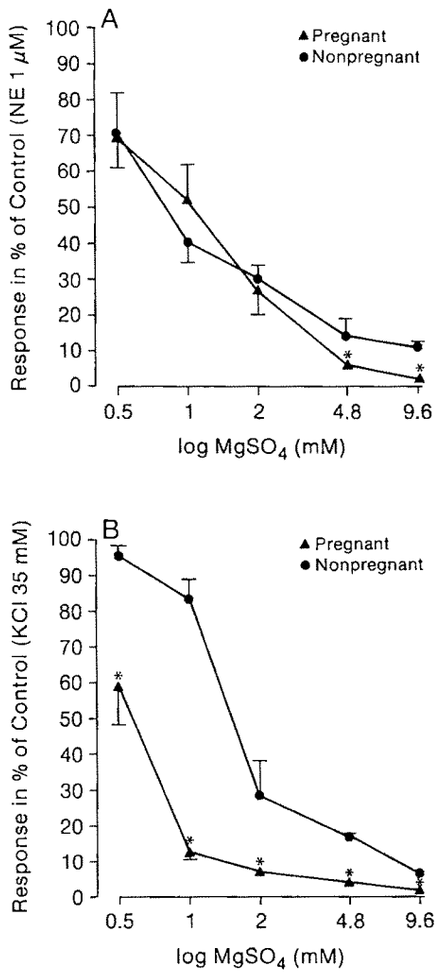


Fig. 2. Concentration-dependent relaxant effects of magnesium sulfate in human uterine arteries contracted by norepinephrine **(A)** and potassium chloride **(B)**. Arteries were from pregnant (▲——▲) and nonpregnant (●——●) patients. Concentrations of magnesium sulfate are those added to Krebs solution containing 1.2 mmol/L magnesium sulfate. Each concentration was given for 10 to 15 minutes. Responses are expressed as percent of control contractile response to norepinephrine (1 μmol/L) **(A)** or potassium chloride (35 mmol/L) **(B)**. Data are means ± SE ($n = 4$ to 9). *Asterisk*, $p < 0.05$.

magnesium sulfate again induced relaxation of the contractions produced by norepinephrine or potassium chloride. Higher concentrations of calcium chloride (3 to 4 mmol/L) were not used in the study because preliminary studies showed that these concentrations inhibited norepinephrine-induced or potassium chloride–induced contractions.

To test for possible mediators of the magnesium sulfate–induced relaxation of uterine arteries, magnesium sulfate (2 mmol/L)–induced inhibition of contractions produced by norepinephrine (1 μmol/L) was

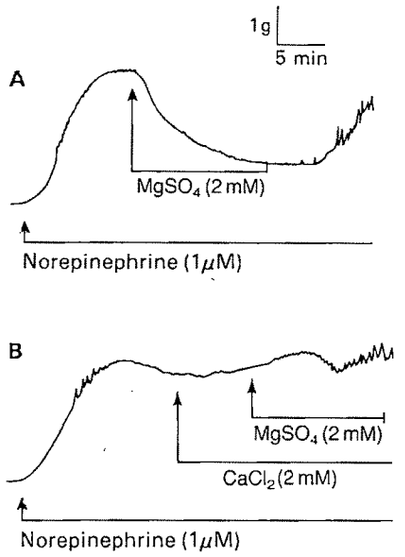


Fig. 3. Calcium chloride antagonism of magnesium sulfate–induced relaxation in uterine artery from a nonpregnant patient. Magnesium sulfate was administered for about 15 minutes in **A** and **B.** Concentration of magnesium sulfate is that added to Krebs solution containing 1.2 mmol/L magnesium sulfate. Concentration of calcium chloride reflects addition of 2 mmol/L calcium chloride to Krebs solution containing 2.5 mmol/L calcium chloride. Preliminary studies showed that 2 mmol/L calcium chloride administered during norepinephrine administration gave the best inhibition of magnesium sulfate–induced relaxation. (Modified from Anesthesiology 1987;67:A636.)

determined in the absence and the presence of indomethacin (5 μmol/L), an inhibitor of cyclooxygenase, methylene blue (10 μmol/L), an inhibitor of soluble guanylate cyclase, and in the presence and then the absence of endothelium. As shown in Fig. 4, the treatments with indomethacin or methylene blue or with removal of the endothelium did not significantly alter the magnesium sulfate–induced relaxation. There was also a lack of effect of these treatments on magnesium sulfate (2 mmol/L)–induced inhibition of contractions produced by potassium chloride or on the potassium chloride–induced contraction itself.

Comment

Our results show that magnesium sulfate acts as a potent dilator in human uterine arteries. Furthermore, we have shown that it is about three times more potent when inhibiting potassium chloride–induced contractions in uterine arteries from pregnant patients than in uterine arteries from nonpregnant patients. At concentrations higher than the value needed for 50% inhibition, magnesium sulfate is also more potent in inhibiting norepinephrine-induced contractions in arteries from pregnant patients than in arteries from nonpregnant patients.

It is recognized that some of the anesthesia-related drugs have prolonged vascular actions, but it was assumed that continuous suffusion of the tissue preparations removed most of the drugs. Furthermore, the fact that the contractile responses to norepinephrine or potassium chloride did not change during the experiments implies either that the anesthesia-related drugs were washed out before commencement of the experiment or that any residual effects of the drugs did not affect the contractile responses to norepinephrine or potassium chloride.

The results show that calcium chloride, at a concentration higher than that in the Krebs solution, almost completely prevented the magnesium sulfate–induced relaxation. These results suggest that magnesium sulfate competes with extracellular calcium for transport into the cell. A caveat to this interpretation of the results is that only single concentrations of calcium chloride and magnesium sulfate were used for the studies. However, our results using human uterine arteries are consonant with the results from numerous other investigations that suggest that magnesium sulfate exerts a relaxing action in blood vessels by interfering with calcium permeability in the cellular membrane.[1-7]

It is well known that contractions of blood vessels induced by potassium chloride (depolarization) are dependent on influx of extracellular calcium through voltage-dependent calcium channels.[14] Organic calcium antagonists, such as verapamil, which block voltage-dependent calcium channels, are especially effective in inhibiting the vasoconstrictor responses to potassium chloride. In this study the findings that magnesium sulfate blocked potassium chloride–induced contractions suggest that magnesium sulfate, like verapamil, acts as a calcium antagonist. It is also well known that contractions induced by norepinephrine are dependent on both release of intracellular calcium and influx of extracellular calcium through receptor-operated and voltage-dependent calcium channels.[14] We have previously reported that verapamil is significantly more potent in inhibiting norepinephrine-induced contractions in arteries from pregnant women than in arteries from nonpregnant women. The results indicated that calcium from extracellular fluid via voltage-dependent calcium channels played a greater role in mediating contraction in arteries from pregnant women,[15] whereas intracellular calcium release played a greater role in mediating contraction in arteries from nonpregnant women. The results of the present study that show no significant difference in the potency for magnesium sulfate acting against norepinephrine-induced contractions in arteries from pregnant and nonpreg-

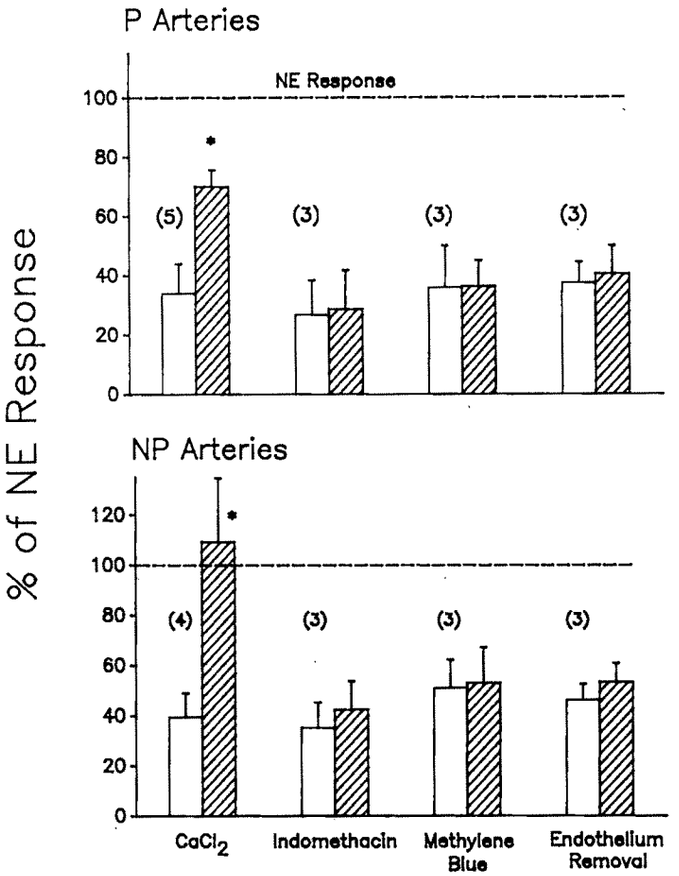


Fig. 4. Effects of various treatments on magnesium sulfate–induced relaxation of contractions produced by norepinephrine *(NE)* in isolated human uterine arteries. *P*, Pregnant; *NP*, nonpregnant. Data are shown as mean ± SE in percent of response to norepinephrine (1 μmol/L) in presence of increased concentration of magnesium sulfate (2 mmol/L) compared with response to norepinephrine (*dotted line*, 100%) in absence of increased concentration of magnesium sulfate. Concentration of 1.2 mmol/L magnesium sulfate is normally present in Krebs solution. *Open bars* represent control magnesium sulfate–induced relaxation of norepinephrine-induced contraction, and *hatched bars* represent magnesium sulfate–induced relaxation in presence of treatment. Treatments included calcium chloride *($CaCl_2$)* (2 mmol/L), indomethacin (5 μmol/L), methylene blue (10 μmol/L), and endothelium removal. Number of experiments is indicated in parentheses. *Asterisk*, Significantly different, $p > 0.05$.

nant patients indicate that magnesium sulfate may interfere with sites involved with intracellular calcium release, as well as with voltage-dependent calcium channels.

Our studies show that magnesium sulfate–induced relaxation does not involve the release of vasodilator prostanoids in the isolated uterine artery. Similar findings have been reported and show that magnesium sulfate–induced dilatation of arterioles or venules in the rat cremaster muscle is not mediated by vasodilator prostanoids.[4] However, other studies report that magnesium sulfate releases vasodilator prostaglandins from endothelial cells of the human umbilical vein[10] and increases the levels of 6-keto-prostaglandin $F_{1\alpha}$ in the urine of humans.[9]

Results from our studies with the use of arterial rings with the endothelium removed or in the presence of methylene blue demonstrate that magnesium sulfate–induced relaxation of uterine arteries from pregnant and nonpregnant patients is independent of the presence of an intact endothelium and does not involve the release of endothelium-derived relaxing factor. Sjogren and Edvinsson[7] also showed that magnesium sulfate–induced relaxation in feline femoral arteries was independent of the presence of endothelium. We have shown previously that the endothelium and

endothelium-derived relaxing factor may indeed play a role in mediating vasodilation induced by drugs such as acetylcholine in uterine arteries from pregnant patients but not in those from nonpregnant patients;[16] whereas nitroprusside, like magnesium sulfate, produces dilation in the presence or absence of endothelium.[12]

Most patients treated with short-term intravenous magnesium sulfate for preeclampsia-eclampsia commonly have plasma magnesium levels (2 to 3 mmol/L or 4 to 6 mEq/L) that are even higher than the 50% inhibition values (0.6 to 1.8 mmol/L or 1.2 to 3.6 mEq/L) for magnesium sulfate determined in our study. Our results suggest that in patients with preeclampsia administration of magnesium sulfate not only helps to prevent convulsions but also causes a dilatation of constricted uterine arteries and thus enhances, or at least maintains, uterine blood flow to the potentially compromised fetus. This suggestion is supported by studies showing that magnesium sulfate produces a slight increase in uteroplacental blood flow in pregnant sheep[1] and causes an increase in uterine artery blood flow, which is secondary to a selective decrease in uterine artery resistance, despite a lack of effect on cardiac output in the pregnant monkey.[2] Our results further suggest that magnesium sulfate, when given as a tocolytic agent to patients in preterm labor, not only relaxes the uterine muscle but also decreases uterine vascular resistance, thereby increasing uterine blood flow.

In summary, our findings indicate that magnesium sulfate, at concentrations that are considered therapeutic, is a direct-acting, endothelium-independent vasodilator of isolated uterine arteries from pregnant and nonpregnant patients.

We acknowledge the late Dr. David Bee for his assistance with the statistics.

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Regulation of the immune response to *Candida albicans* by monocytes and progesterone

Aliza Kalo-Klein, PhD, and Steven S. Witkin, PhD
New York, New York

Recurrent vaginal infections caused by *Candida albicans* are associated with decreased cell-mediated immune responses. We report that circulating progesterone levels and variations between persons in the activity of their monocytes are two of the factors that influence the extent of lymphocyte proliferation in response to *C. albicans*. With the use of peripheral blood mononuclear cells from five men and four women, removal of the monocytes increased the lymphocyte response to *C. albicans* antigens in eight persons. The percentage increase in proliferation was inversely proportional to the proliferative response observed when the monocytes were present, suggesting that differences existed between persons in the ability of their monocytes to down regulate the response. An approximate 50% decrease in *C. albicans*–induced lymphocyte proliferation was observed in the presence of luteal-phase levels (25 ng/ml), as opposed to proliferative-phase levels (0.15 ng/ml) of progesterone. Monocyte removal obviated the ability of 25 ng/ml progesterone to inhibit this response, suggesting that progesterone inhibited lymphocyte proliferation through a monocyte-dependent mechanism. Thus fluctuations in a woman's monocyte activity in response to genetic, hormonal, or environmental factors may influence her ability to mount an effective cell-mediated immune response to *C. albicans*. (AM J OBSTET GYNECOL 1991;164:1351-4.)

Key words: *Candida albicans,* progesterone, monocytes, vaginitis, cell-mediated immunity

The proliferation of *Candida albicans* in the human vagina is inhibited primarily if not entirely by the cell-mediated immune system.[1] Defects in cell-mediated immunity[2, 3] and abnormal monocyte responses[4] have been associated with recurrences of *Candida* vaginitis. Specifically, the abnormal production of prostaglandin E_2 in response to *C. albicans* by monocytes from women susceptible to recurrent vaginitis leads to an inhibition of the immune response which limits the growth of this organism.[4] Furthermore, prostaglandin E_2 also promotes the formation of germ tubes by yeast forms of *C. albicans,*[5] thereby providing the organism with the capacity to invade mucosal epithelia[6] and initiate a clinical infection. Germ tube formation is inhibited by interferon-γ,[5] a product of activated T-lymphocytes.

Recent evidence has also shown that the cellular immune response to *C. albicans* is decreased during the luteal phase of the menstrual cycle, coinciding with the time when the incidence of *Candida* vaginitis is increased.[7] This indicates that fluctuations in hormone levels may also modulate susceptibility to *Candida* infection.

In this investigation, we examined directly the effects of monocyte removal and progesterone addition on the in vitro lymphocyte proliferative response to *C. albicans.*

From the Immunology Division, Department of Obstetrics and Gynecology, Cornell University Medical College.
Received for publication September 18, 1990; revised December 5, 1990; accepted December 28, 1990.
Reprint requests: Steven S. Witkin, PhD, Cornell University Medical College, Department of Obstetrics and Gynecology, 525 E. 68th St., New York, NY 10021.
6/1/27674

Material and methods

Subjects. Subjects were five men and four women of reproductive age with normal 28- to 30-day menstrual cycles, no history of *Candida* vaginitis, and negative for *Candida* on vaginal cultures. All were in good health and not taking any medication at the time of the study. Two of the women were in the second week and two were in the fourth week of their cycles.

Peripheral blood mononuclear cells. Peripheral blood mononuclear cells were isolated from heparinized blood by Ficoll-Hypaque gradient centrifugation. Monocytes were removed by adherence to plastic dishes as described previously.[4] Briefly, peripheral blood mononuclear cells (5×10^6/ml) in Roswell Park Memorial Institute 1640 medium containing 10% heat-inactivated autologous serum, were plated on 60 mm^2 plastic Petri dishes, incubated at 37° C in a 5% carbon dioxide incubator for 60 minutes, and the nonadherent cells removed with a sterile pipette. Monocyte contamination of the nonadherent cell fraction was routinely <5%.

Lymphocyte proliferation. To measure lymphocyte proliferation, 2×10^5 peripheral blood mononuclear cells or nonadherent cells were added to wells of a microtiter plate in Roswell Park Memorial Institute 1640 medium containing 25 mm/L HEPES buffer, L-glutamine, penicillin (100 U/ml), streptomycin (100

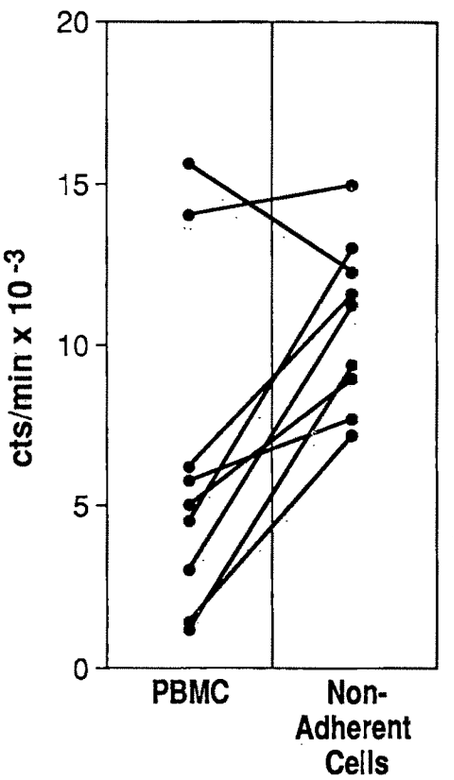


Fig. 1. Effect of monocyte removal on the lymphocyte proliferative response to *C. albicans*. Peripheral blood mononuclear cells *(PBMC)* were isolated from nine persons and monocytes were removed from an aliquot of each by adherence to plastic. Peripheral blood mononuclear cells or nonadherent cells were incubated for 7 days in the presence of *C. albicans* antigens and lymphocyte proliferation was measured.

μg/ml), kanamycin (100 μg/ml), and 10% autologous serum. The final volume was 0.2 ml. Triplicate wells received either no addition, 32 protein nitrogen units/ml of *C. albicans* antigens (Hollister-Stier, Spokane, Wash.), 0.15 ng/ml progesterone with or without *Candida* antigens, or 25 ng/ml progesterone with or without *Candida* antigens. After 6 days in a 37° C, 5% carbon dioxide incubator, tritium-thymidine (0.2 μCi/well) was added. After an additional 18 hours, the cultures were harvested onto glass fiber filters, washed, and incorporated radioactivity was determined.[7] The triplicate determinations were averaged and the mean value is reported. Radioactivity incorporated in the absence of *Candida* antigens or progesterone was subtracted from the experimental values.

Statistics. The relationship between the immune responses of peripheral blood mononuclear cells and nonadherent cells to *Candida* was evaluated by the Spearman rank correlation test.

Table I. Effect of proliferative-phase levels of progesterone on the proliferation of lymphocytes from men in response to *Candida*

	Proliferation (counts per minute × 10^3)		
Donor	*Control*	*Progesterone*	*% Control*
1	5.8	8.8	150.7
2	13.9	18.5	132.7
3	15.6	19.7	126.3
4	5.0	5.2	104.6
5	2.9	5.7	198.4

Progesterone was added at 0.15 ng/ml.

Table II. Effect of luteal-phase levels of progesterone on the proliferation of lymphocytes from men in response to *Candida*

	Proliferation (counts per minute × 10^3)		
Donor	*Control*	*Progesterone*	*% Control*
1	5.8	4.3	74.0
2	13.9	10.6	76.3
3	15.6	8.9	57.3
4	5.0	3.8	76.1
5	2.9	2.1	74.9

Progesterone was added at 25 ng/ml.

Results

Effect of monocyte removal on the immune response to *Candida*. Lymphocytes from each of the five men and four women proliferated in the presence of *C. albicans* (Fig. 1). The two women in the proliferative phase of their cycles had higher proliferative responses (6140 and 4576 counts per minute) than did the two women from whom samples were obtained during the luteal phase (1406 and 1246 counts per minute). The lymphocyte responses of the men were each at least double the values obtained with lymphocytes from the women in the luteal phase; responses of three of the men were comparable to the results with proliferative phase–derived lymphocytes from the two women.

Except for the one man with the highest recorded level of radioisotope incorporation, monocyte removal led to a marked increase in lymphocyte responsiveness (Fig. 1)

The magnitude of the increased response after monocyte removal was inversely proportional to the initial response to *Candida* ($p < 0.001$) in both men and women (Fig. 2); that is, those persons whose peripheral blood mononuclear cell response to *Candida* was

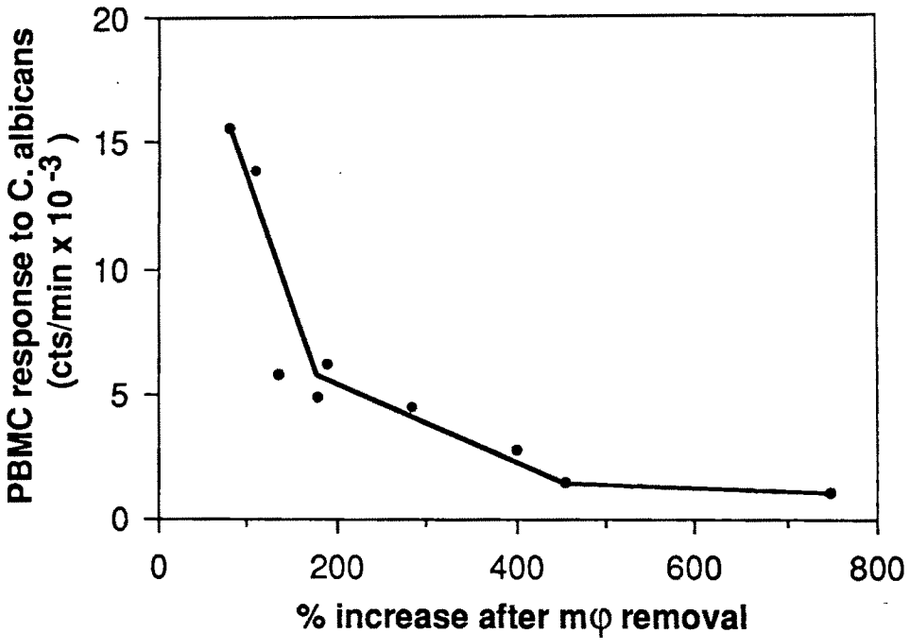


Fig. 2. Relation between the peripheral blood mononuclear cell *(PBMC)* response to *C. albicans* and the percentage increase in lymphocyte response after monocyte removal. The effect of monocyte *(mϕ)* removal on *Candida*-induced tritium-thymidine incorporation by lymphocytes from nine persons was determined.

lowest exhibited the largest increase in lymphocyte proliferation when the monocytes were removed.

Effect of progesterone on the immune response to *Candida*. The effect of exogenous progesterone on the ability of lymphocytes from the five male subjects to respond to *Candida* was evaluated. In the presence of 0.15 ng/ml progesterone, the level found during the proliferative phase of the menstrual cycle,[7] there was a modest stimulation of lymphocyte proliferation in four of the five men (Table I). In marked contrast, increasing the progesterone concentration to the level present during the luteal phase (25 ng/ml) resulted in a modest inhibition of lymphocyte proliferation in all five men (Table II). Comparison of radioisotope incorporation at the two progesterone concentrations makes it apparent that there is an approximate 50% difference in lymphocyte proliferation in the presence of proliferative-phase or luteal-phase levels of this hormone.

Removal of the monocytes completely obviated the ability of 25 ng/ml progesterone to inhibit the immune response to *Candida* in each of the five men (Table III). Lymphocyte proliferation increased to a level 136% ± 32% of that observed by nonadherent cells incubated in the absence of progesterone. Similar to our earlier observations, the percentage increase in radioisotope incorporation after monocyte removal was inversely proportional to the level of incorporation obtained in the presence of monocytes ($p < 0.001$).

Table III. Effect of monocyte removal on the ability of progesterone to inhibit the lymphocyte proliferative response to *Candida*

	Proliferation (counts per minute × 10^3)		
Donor	*PBMCs*	*Nonadherent cells*	*% Increase*
1	4.3	11.3	262.8
2	10.6	17.9	168.9
3	8.9	20.5	230.3
4	3.8	14.2	373.7
5	2.1	10.2	485.7

PBMC, Peripheral blood mononuclear cells. Progesterone was present at 25 ng/ml.

Comment

Removal of monocytes from the peripheral blood mononuclear cells of eight of nine men and women enhanced *C. albicans*–induced lymphocyte proliferation. Furthermore, the percentage increase was inversely proportional to the level of proliferation ob-

served in the presence of monocytes. Inhibition of the cellular immune response to *C. albicans* by luteal-phase levels of progesterone appeared to be similarly regulated by monocytes. In the absence of monocytes, progesterone was no longer immunosuppressive and the percentage increase was also inversely proportional to the level of proliferation in the presence of monocytes. Thus individual differences in the ability of monocytes to regulate lymphocyte proliferation is a major determinant of the extent of cell-mediated immune system activation induced by *C. albicans*. Monocyte-dependent variations in the extent of lymphocyte proliferation between persons has been observed previously.[8] Perhaps monocytes from each subject in this study differed in their capacity to differentiate into macrophages, process *Candida* antigens, or present them in association with major histocompatibility class II antigens to T-lymphocytes. Alternatively, quantitative difference in the production of monocyte products such as interleukin-1 or prostaglandin E_2 would also be expected to influence the extent of lymphocyte proliferation. Experiments to differentiate between these possibilities are in progress.

The observation that low levels of progesterone stimulate whereas high levels inhibit lymphocyte proliferation are in agreement with a previous report[9] that showed that low concentrations of progesterone stimulated production of interleukin-1 by monocytes; at higher progesterone levels, interleukin-1 release was significantly decreased. Interleukin-1 is required for the induction of lymphocyte proliferation.[10]

In contrast to the effect of luteal-phase levels of progesterone on *Candida*-induced lymphocyte proliferation, proliferative-phase or luteal-phase levels of estrogen were not inhibitory (data not shown). Thus fluctuations in progesterone levels during the menstrual cycle appear to be responsible for the inhibition of cell-mediated immunity[7] and the onset of *Candida* vaginitis during the luteal phase. Progesterone-induced immune suppression has been observed previously. In two studies[11, 12] progesterone inhibited early events in lymphocyte proliferation, suggestive of an effect on monocytes. In a third study,[13] however, the kinetics of progesterone-induced inhibition suggested a direct effect of the hormone on T-lymphocytes. This study supports the two former studies that indicate the existence of a monocyte-dependent step in progesterone-mediated immunosuppression.

In general, a variety of experimental evidence[1, 4, 14] suggests that the degree of susceptibility of women to contracting a *Candida* vaginal infection may be influenced by alterations in monocyte activity induced by genetic, environmental, and hormonal factors. Identification of highly susceptible women and development of techniques to augment their cellular immune responses to *Candida* may ultimately be beneficial to reduce the frequency of recurrences of *Candida* vaginitis.

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Regulation of CA 125 production by amnion and WISH cells in culture

Antonella Barbati, PhD, Lorena Broccucci, PhD, Maurizio M. Anceschi, MD, PhD, and Ermelando V. Cosmi, MD

Perugia, Italy

Amnion and human amnion–derived WISH cells release CA 125 antigen into culture medium. CA 125 output was higher in WISH cells than in amnion cells. In this study we showed that release of the antigen is regulated, with both amnion and WISH cells responding in a similar manner to the tested agents. Release of CA 125 decreased in the presence of dexamethasone (10^{-6} mol/L) or cycloheximide (0.1 μg/ml) and increased when colchicine (0.01 μg/ml) was added to the culture medium. Stimulatory and inhibitory effects were more apparent after 3 days in culture. The precise mechanisms by which some of these agents (colchicine and dexamethasone) affect CA 125 release remain unknown. We propose that amnion and WISH cells in culture represent a useful model to investigate some regulatory aspects of the production of CA 125 in normal tissues. (Am J Obstet Gynecol 1991;164:1355-9.)

Key words: CA 125 release, amnion, WISH cells, colchicine

The cancer antigen CA 125, first found to be elevated in >80% of epithelial ovarian carcinomas[1] and in a minor percentage in a variety of nonmalignant diseases,[2,3] has also been detected in normal biologic fluids such as human milk,[4] cervical mucus,[5] seminal plasma (unpublished data), serum of pregnant women in the first trimester of gestation, and in amniotic fluid.[6,7] Furthermore, CA 125 was detected in fetal müllerian duct derivatives, fallopian tube, endometrium, endocervix, pleura, pericardium,[8] and recently in placental tissues,[6,9,10] suggesting that CA 125 may be a secretory product of many normal human epithelia. In a recent study,[11] we reported that CA 125 was detectable in human amnion cells in primary culture and in their culture medium, in which the antigen accumulated as a function of time.

A poorly investigated aspect is the regulatory pattern of cellular expression of CA 125. In vitro it has been shown that CA 125 expression by ovarian carcinoma cell lines is modulated by certain agents,[12,13] whereas in vivo serum levels of CA 125 seem to respond to hormonal stimulation.[14]

From the Second Department of Obstetrics and Gynecology, Policlinico Monteluce.
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Reprint requests: A Barbati, PhD, Second Department of Obstetrics and Gynecology, Policlinico Monteluce, Perugia, Italy 06100.
6/1/27102

To elucidate some of the mechanisms involved in the regulation of CA 125 expression in vitro, this study was undertaken to evaluate whether CA 125 antigen production or release by amnion and WISH cells, a human amnion–derived cell line that retains some of the characteristics of amnion epithelium,[15,16] is a modulated process.

Material and methods

Cell cultures

Amnion cells. Primary cultures of human amnion cells were prepared from term placentas obtained at the time of elective cesarean section, as previously described.[11] The cells, approximately 40,000 cells/well per 0.2 ml, were seeded in 96 sterile flat-bottom well (0.6 cm diameter) plates (Linbro, Flow Laboratories, Irvine, United Kingdom), and grown in a mixture of Dulbecco's modified Eagle's medium and nutrient mixture F-12 Ham. The cells became confluent within 10 to 14 days. Confluent cultures were used for all experiments, which were conducted with replicates of three wells of amnion cells.

WISH cells. The human amnion–derived WISH cell line (ATCC CCL25) was obtained from the American Type Culture Collection, Rockville, Md. Approximately 20,000 cells/well in 0.2 ml were subcultured in 0.6 cm diameter wells in minimum essential medium with Earle's salts and L-glutamine (2 mmol/L), penicillin (100 U/ml), streptomycin (100 μg/ml), and heat-inactivated newborn calf serum (10% vol/vol) were added. Maintained at 37° C in an atmosphere of air and carbon dioxide (5%), the cells routinely attained confluence within 72 hours.

Treatments. To normalize the difference in growth

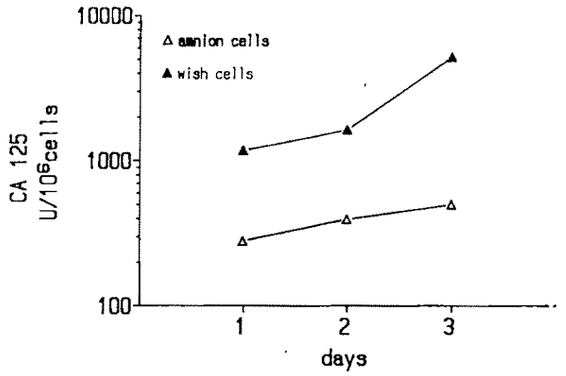


Fig. 1. Comparison of CA 125 accumulation into the medium of amnion and WISH cells in culture as function of time (days post confluence). Each point represents the mean of three replicates from two different experiments. Note logarithmic scale.

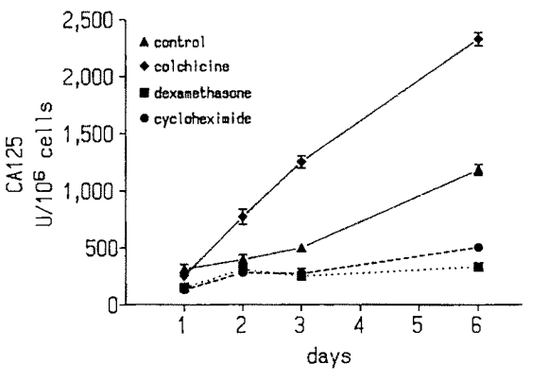


Fig. 2. Effect of colchicine (0.01 μg/ml), dexamethasone (10^{-6} mol/L), cycloheximide (0.1 μg/ml) on CA 125 accumulation into the medium of amnion cells in primary culture. At 3 and 6 days post confluence, difference between control and treatments was statistically significant by analysis of variance ($p < 0.05$). Dexamethasone vs cycloheximide, NS.

rates between control and treated cells, all experiments were conducted with confluent monolayers of amnion or WISH cells (confluence being evaluated by inspection of the culture under inverted microscopy). At confluence (day 0) each well was washed once and fresh medium was added with three replicates for each experimental condition.

CA 125 production was determined after incubation of cells in medium alone (control) and medium with the following substances added: dexamethasone (10^{-6} mol/L), colchicine (0.01 μg/ml), cycloheximide (0.1 μg/ml), actinomycin (0.012 μg/ml) and tunicamycin (1.25 μg/ml), purchased from Sigma Chemical Co., St. Louis. These concentrations were selected because they were the highest that did not affect cell viability, as evaluated by trypan blue exclusion in preliminary experiments.

Collection of samples. The medium was not changed until collection at days 1, 2, 3, and 6 for amnion cells, and at days 1, 2, and 3 for WISH cells. Harvested medium was centrifuged at 3000 *g* for 10 minutes to remove cells and debris and stored at −20° C until assayed.

CA 125 assay. CA 125 was assayed by an immunoradiometric kit (Sorin Biomedica, Saluggia, Italy). Dilutions of samples (1:5 to 1:10) were used for the determination of CA 125 in medium from WISH cells because levels often exceeded 500 U/ml (the highest point of the standard curve). Dilutions were made with minimum essential medium.

Cell count and protein content. To normalize CA 125 data to cell number and cell protein content, cell monolayers were detached at 37° C with a solution of 0.25% trypsin (wt/vol) and 0.02% ethylenediaminetetraacetic acid (EDTA) at the time of medium collection and counted with a hemacytometer chamber. For determination of protein content, the cells were washed once and suspended in sonication buffer (Tris hydrochloride 12 mmol/L, EDTA 10 mmol/L, sodium chloride 100 mmol/L, Triton X-100 0.1%). Cells were then disrupted by sonication (three bursts of 5 seconds each) and protein content was measured with the Bio-rad assay (Bio Rad Laboratory, Muenchen, Federal Republic of Germany).

Statistical analysis. Statistical differences were assessed with the Student *t* test for comparison of CA 125 release by amnion and WISH cells and by one-way analysis of variance for treated and control cells. The experimental conditions were repeated with replicates of three wells for cultures of amnion and WISH cells.

Results

Comparison of CA 125 release by amnion and WISH cells. At 1, 2, and 3 days post confluence, the medium from WISH cells in culture contained a higher amount of CA 125 than did medium from amnion cells with a median value of 1070, 2970, and 5900 U/10^6 cells for WISH cells, respectively, *vs* 270, 390, and 500 U/10^6 cells for amnion cells, respectively (Fig. 1). Both of them continuously produced and released CA 125, as demonstrated by the pattern of accumulation in culture medium. A lag period of about 2 hours occurred to found levels of CA 125 immunologically detectable (data not shown). Amnion and WISH cells maintained in serum-free medium produced less CA 125 than that of cells maintained in medium with serum added (data not shown).

Regulation of CA 125 production in amnion cells. The amount of CA 125 released by the primary monolayer culture of amnion cells treated with dexametha-

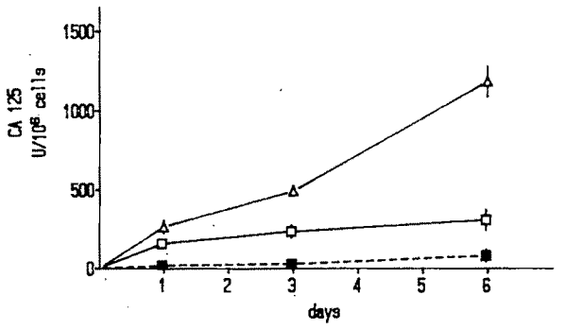


Fig. 3. Effect of dexamethasone added after confluence *(open square, solid line)* and immediately during culture set up *(close square, dashed line)* on release of CA 125 by amnion cells in culture *(open triangles, solid line)* as function of time. Replicates of three wells (dexamethasone vs dexamethasone added at set up, $p < 0.05$).

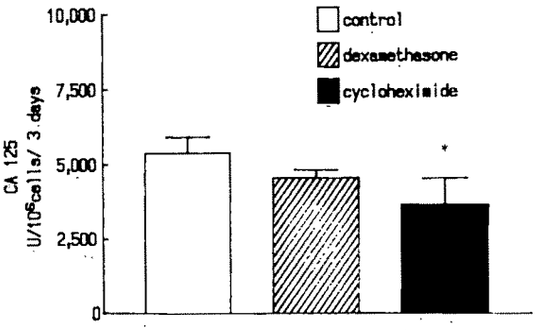


Fig. 4. Effect of dexamethasone (10^{-6} mol/L) and cycloheximide (0.1 μg/ml) on CA 125 release by WISH cells after 3 days in culture post confluence. Replicates of three wells from four experiments (mean ± SEM). *Asterisk*, $p < 0.05$ vs control.

Table I. Total cell number ($\times 10^{-5}$) per well and percentage viability after different periods in culture in a typical experiment with amnion and WISH cells

	Amnion cells				*WISH cells*	
	3 days		*6 days*		*3 days*	
Treatment	*No.*	*%*	*No.*	*%*	*No.*	*%*
Control	310 ± 14	98	268 ± 39	95	1200 ± 141	95
Colchicine	362 ± 59	97	374 ± 8	96	385 ± 21	90
Dexamethasone	400 ± 76	98	407 ± 38	96	986 ± 76	96
Cycloheximide	341 ± 13	97	297 ± 18	94	1415 ± 106	94

Values are means ± SDs of replicates of three wells.

sone or cycloheximide was reduced by about 50% at day 3 post confluence (suppressor index, 50% for dexamethasone; 46% for cycloheximide). In treated cultures at 6 days post confluence, the suppressor index rose to 72% for dexamethasone and 58% for cycloheximide.

By contrast, colchicine acted as an inducer of CA 125 production (stimulator index, 1.5 and 2 at days 3 and 6 post confluence, respectively; Fig. 2).

Total cell number and viability as assessed by trypan blue exclusion was not affected by treatment with the various substances studied (Table I).

When amnion cells were directly seeded in the presence of dexamethasone (10^{-6} mol/L) and CA 125 measured at various periods post confluence, treatment with dexamethasone resulted in an enhanced inhibitory effect (93%) on CA 125 release from day 1 onward (Fig. 3).

Regulation of CA 125 production in WISH cells. The same experiments performed with WISH cells for release of CA 125 indicated a response pattern similar to that of amnion cells. The amount of CA 125 released by WISH cells treated with dexamethasone 10^{-6} mol/L tended to be lower at day 3, although not statistically significant. Cycloheximide significantly inhibited CA 125 production, causing a 33% decrease in basal output (Fig. 4). Incubation of cells in the presence of colchicine induced CA 125 release with levels increasing about sixfold after 3 days in culture (Fig. 5). Exposure to colchicine prevented cell proliferation, which usually occurs in WISH cells even after confluence, so that total cell number, but not viability, was reduced (Table I). Colchicine was effective in the stimulation of CA 125 release by WISH cells in serum-free medium (data not shown). Actinomycin and tunicamycin treatments did not have significant effects on CA 125 production after 3 days. When CA 125 data were normalized to cell protein content, the response pattern was similar to that observed per 10^6 cells.

Comment

In this study we used human amnion cells in primary monolayer culture as a model system for the study of the regulation of CA 125 synthesis in vitro. It was demonstrated that the production of CA 125 was stimulated by colchicine and inhibited by dexamethasone and cycloheximide. Further, CA 125 production by the hu-

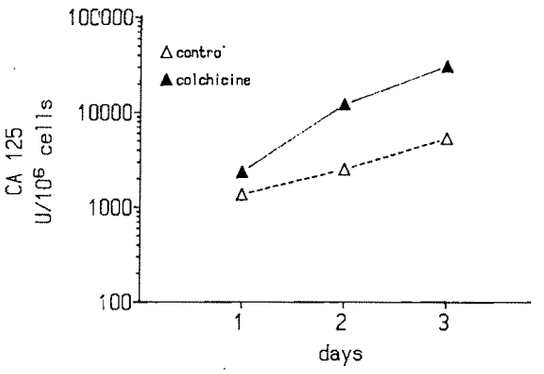


Fig. 5. Effect of colchicine (0.01 μg/ml) *(closed triangles, solid line)* on CA 125 accumulation into culture medium of WISH cells with respect to control *(open triangles, dashed line)* as function of time (days post confluence). Each point represents the mean of replicates of three from four different experiments. Note logarithmic scale.

man amnion–derived WISH cell line was modulated in a manner similar to that observed in primary cultures of human amnion.

In the presence of inhibitors of protein synthesis, production of CA 125 by these cells was inhibited. Cycloheximide, a potent translation inhibitor, reduced CA 125 output from amnion and WISH cells in culture, providing evidence that intact protein synthesis is required for CA 125 release. However, the output of the antigen did not seem to be completely suppressed, suggesting the existence of intracellular stores of the glycoprotein, which continues to be released at a slower rate even in the presence of inhibition of protein synthesis. Although it is not possible to conclude that CA 125 is synthesized again by these cells, the release pattern and inhibition in the presence of cycloheximide is highly suggestive of such a possibility.

Dexamethasone was shown to decrease CA 125 expression more effectively in amnion than in WISH cells in culture. The effect was similar to analogous studies performed on ovarian carcinoma cell lines. It has been reported that OVCA-433 (a human ovarian carcinoma cell line) contains glucocorticoid receptors with high affinity for dexamethasone and that treatment with dexamethasone markedly inhibits (90%) the release and surface density of CA 125 by the same cell line.[12, 17] The higher inhibitory effect of dexamethasone on CA 125 release by OVCA-433, as compared with the results of this study, is probably related to the fact that a longer period of incubation (7 days) was used and dexamethasone was added at the time of cell seeding.

The mechanisms by which dexamethasone depresses CA 125 expression are still unclear. As suggested by Karlan et al.,[12] dexamethasone could directly inhibit the production of CA 125 at the level of protein synthesis, or it could alter the protein conformation or glycosylation states, which would in turn destroy or block the CA 125 determinant. Alternatively, dexamethasone might induce either a protein, which binds to the glycoprotein and blocks the CA 125 determinant, or an enzyme, which specifically destroys or alters the glycoprotein. The reported difference in dexamethasone inhibition of CA 125 between amnion and WISH cells may be because of a different number of receptors for dexamethasone.

After incubation with colchicine, release of CA 125 was markedly induced both in amnion and WISH cells in culture in a time-dependent fashion. The increased CA 125 shedding into culture medium in the presence of colchicine was not associated with increased cell lysis because cell viability in treated and control cultures was not affected with the concentrations of colchicine used in the experiments, as determined by trypan blue exclusion (>90% in both control and treated cells). The effect of colchicine on WISH cells, which unlike amnion cells tend to proliferate even after reaching confluence, seemed to be cytostatic rather than cytolytic.

Colchicine, a known antimitotic drug, is known to bind to tubulin, inducing a conformation change of the protein, which causes an alteration in the assembly of cell microtubules.[18] In unrelated experiments on isolated type II pneumonocytes, colchicine has been shown to stimulate basal secretion of surfactant, which involves microtubular structures.[19, 20] How alteration of cytoskeleton elements might mediate CA 125 production or release remains to be elucidated, although it is tempting to speculate that colchicine acts on secretion rather than on production of the glycoprotein.

Induction of CA 125 in vitro has also been reported in an ovarian carcinoma cell line (HTB-77) treated with interferon-γ, the effect of which was time-dependent and maximal after 3 days in culture.[13] Nonetheless, our study is the first to describe an induction of CA 125 in normal tissue in vitro. Although amnion cells are clearly not neoplastic, their marked proliferation during pregnancy suggests the presence of a common cellular mechanism that regulates antigen production in rapidly proliferating cells.

The involvement of ribonucleic acid synthesis in CA 125 output was studied with actinomycin D, a ribonucleic acid polymerase inhibitor. In this study treatment with actinomycin D for 3 days did not affect significantly CA 125 output in WISH cells. The fact that actinomycin does not interfere with CA 125 accumulation may be consistent with a mechanism in which CA 125 production is regulated at a posttranslational level. Alternatively, the putative messenger ribonucleic acid for the protein may have a long half-life or be particularly stable.

Tunicamycin, an inhibitor of protein glycosylation, did not have any significant effect on CA 125 expres-

sion on WISH cells, suggesting that the antigenic determinant is not affected by inhibition of protein glycosylation.

The findings of this study are several: (1) amnion and WISH cells produce CA 125; (2) the output is higher in WISH cells than in amnion cells; (3) several agents modulate CA 125 release in amnion and WISH cells in a similar manner; (4) CA 125 output is a dynamic and regulated process. On the basis of the findings of this investigation, we propose that amnion cells maintained in monolayer culture represent an appropriate model for the study of the regulation of CA 125 biosynthesis in this tissue. In addition, amnion and WISH cells can be used by many investigators to study specific aspects of the regulation of oncofetal antigens in amnion and in tumor tissues.

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Corrections

In the article by Silver et al., entitled "Risk factors for perinatal group B streptococcal disease after amniotic fluid colonization" (Am J Obstet Gynecol 1990;163:19-25), in Table V, on page 22, under *Bacteremia,* for Serotype Ia the mean antibody level of 80.6 was printed with a standard deviation of 55.5. The correct mean is 80.6 but the standard deviation should be omitted.

In the Reply by Johnson and Richards (Am J Obstet Gynecol 1990;163:1364), in line 28, "pH 7.21" should have been "pH 7.12."

LETTERS TO THE EDITORS

Anesthesia and funic acid-base values

To the Editors: I read with great interest the article by Shyken et al. (Shyken JM, Smeltzer JS, Baxi LV, Blakemore KJ, Ambrose SE, Petrie RH. A comparison of the effect of epidural, general, and no anesthesia on funic acid-base values by stage of labor and type of delivery. AM J OBSTET GYNECOL, 1990;163:802-7). Several points regarding their method and results deserve further comment.

First, the authors place considerable emphasis on their finding of significantly lower umbilical arterial and venous partial pressure of oxygen (P_{O_2}) values in the infants who were born during epidural anesthesia versus those born during general anesthesia for cesarean delivery. However, rather than dwelling on the lower P_{O_2} values in the epidural anesthesia group, the authors should have emphasized that the general anesthesia group had *higher* P_{O_2} values, a result that is easily explained by the fact that administration of general anesthesia usually includes 30% to 50% fraction of inspired oxygen (via endotracheal tube and positive-pressure ventilation) after preoxygenation with 100% oxygen.[1] It is unlikely that maternal fraction of inspired oxygen (and thus maternal P_{O_2}—a parameter that is not mentioned by Shyken et al.) was comparable in the general anesthesia group versus epidural anesthesia group during cesarean delivery. Moreover, by what mechanism do the authors propose that epidural anesthesia can lower neonatal P_{O_2} values? Their statement, "Another reason for relative hypoxemia and hypercarbia associated with the use of epidural analgesia is hypotension as a result of sympathetic blockade" is made without reference. The article by Brizgys et al.[2] (which is referred to by the authors) found only a slightly lower pH (7.31 vs 7.33) when hypotension occurred during cesarean delivery; P_{O_2} and partial pressure of carbon dioxide values were unaffected.

Second, the acid-base values of the neonates who were born by vaginal delivery are claimed by the authors to be attributable to the longer labors of these women. Considerable controversy exists regarding the acid-base sequelae of long labors.[3] This retrospective study is subject to all of the selection biases that flaw the numerous other retrospective studies that correlate the length of labor with the use of epidural anesthesia. One wonders if the results of Shyken et al. would have been different if all of the patients in the vaginal delivery group had been randomized to receive either epidural anesthesia or no anesthesia during labor? As stated in a recent letter to this JOURNAL,[4] the time has come for a prospective, randomized trial of epidural anesthesia versus no epidural anesthesia during labor. (However, it would appear difficult to ethically design and execute such a study.)

Finally, and most important, is that the study by Shyken et al. makes no mention of maternal risks of general anesthesia versus epidural anesthesia. A recent review discusses the inherent risks of general anesthesia for the parturient, in which all anesthetic-related deaths in North Carolina over a 40-year period were attributable to complications from general anesthesia.[5] Another similar review in Massachusetts found that the vast majority of anesthetic-related deaths over a 30-year period were related to general anesthesia, and all such deaths over the last decade were a result of general anesthetic complications.[6] At our institution, we perform more than 2500 cesarean deliveries per year; our rate of use of general anesthesia has been steadily declining over the last two decades and is currently about 8%. We are proud of this accomplishment. Shyken concludes by stating that his results are "unlikely . . . to be clinically important for the term fetus with a normal electronic monitor pattern." We quite agree, and therefore other factors must be considered when an anesthetic technique is chosen. If the data by Shyken et al. are ever used to discourage a woman from having epidural anesthesia during labor or conversely, to encourage the use of general anesthesia for cesarean delivery, then a great disservice will have been done, not only to the obstetric and anesthetic communities, but especially to the women and infants who seek our expertise in obtaining safe and comfortable childbirth.

William R. Camann, MD
Division of Obstetric Anesthesia, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St., Boston, MA 02115

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Reply

To the Editors: We appreciate the interest expressed by Dr. Camann regarding our article. It is generally accepted that a difference in fetal partial pressure of oxygen may be based on maternal administration of oxygen. The hypothesis that preoxygenation alone explains the higher partial pressure of oxygen in the general anesthesia group can be tested by factor analysis for the independent effects of respiratory function,

metabolic function, and oxygenation. Such analyses of our data reveal that respiratory function is higher with vaginal delivery versus cesarean section and with no anesthesia versus epidural anesthesia and that with cesarean, there is no difference between epidural and general anesthesia. Metabolic differences are small between groups, but reserve was lower with labor compared with no labor and with no anesthesia compared with epidural anesthesia. Oxygenation was higher with general anesthesia versus epidural anesthesia, but only during labor.

A decrease in mean maternal arterial blood pressure during conduction anesthesia reduces uterine blood flow proportionately[1, 2] and has the potential for fetal compromise. Our study demonstrated no significant relationship between hypotension and acid-base parameters.

In spite of the contention that controversy exists regarding the acid-base sequelae of length of labor,[3, 4] our data demonstrate longer durations of stage I and stage II in women who have epidural analgesia compared with women who do not have it. This protraction is independent of parity and explains the decreases in pH and venous partial pressure of oxygen in women who delivered vaginally with epidural anesthesia. It is possible that women who had "more difficult labors" were selected for epidural anesthesia by their physicians. We understand the limitations of any retrospective study. However, a prospective, randomized trial does not appear to be forthcoming.

Last, the data that are presented in our article are not intended to disparage the use of epidural analgesia-anesthesia for labor or cesarean section; to do so would represent gross overinterpretation of our results. The intent was not to compare maternal morbidity with general or epidural anesthesia. Rather, the purpose, as stated, was to investigate the premise that epidural analgesia, when compared with no anesthesia or general anesthesia, was advantageous *to the fetus*. In fact, we found no such advantage in the healthy fetus, born of a normal pregnancy at the end of labor, by cesarean section during labor, or by elective cesarean section. We did not generalize to other populations or otherwise indict epidural anesthesia. The highly important maternal considerations of safety and comfort are entirely different issues that are intentionally not addressed in this study.

Jaye M. Shyken, MD, James S. Smeltzer, MD, and Roy H. Petrie, MD, ScD

Department of Obstetrics and Gynecology, Washington University School of Medicine, 5535 Delmar Blvd., St. Louis, MO 63112

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Embryoscopy: Old technique new for the 1990s?

To the Editors: With great interest I read the article by Cullen et al. (Cullen MT, Reece EA, Whetham J, Hobbins JC. Embryoscopy: Description and utility of a new technique. AM J OBSTET GYNECOL 1990;162:82-6). The authors did not point out that all the previous authors cited in the references incorrectly believed the chorionic membrane has transparent areas through which visualization of the embryo is possible. They did not understand that only chorion perforation makes embryo observation possible and therefore they did not continue with their investigations.

My experience in embryoscopy is similar to that of Cullen et al. and I can confirm that only perforation of the chorion allows the visualization of the embryo. In 1984 I investigated the possibility of whole embryo visualization in the first series of 100 cases between 8 and 13 weeks' gestation. The study was conducted without ultrasonographic guidance immediately before suction curettage in women undergoing voluntary abortion. I used an endoscope with an outer diameter of 4 mm and a length of 30 cm. In 75% of the cases visualization of the whole embryo was achieved. In two patients a diagnostic attempt to continue the pregnancies was successfully performed. The pregnancies continued uneventfully and reached term. The children are now 6 and 5 years old, respectively.[1]

In 1985 I performed embryoscopy in another series of 62 cases between 7 and 12 weeks' gestation. I used the optic of the Chorionscope (length, 30 cm; outer diameter, 2.7 mm; angle, 70 degrees) (Richard Wolf,

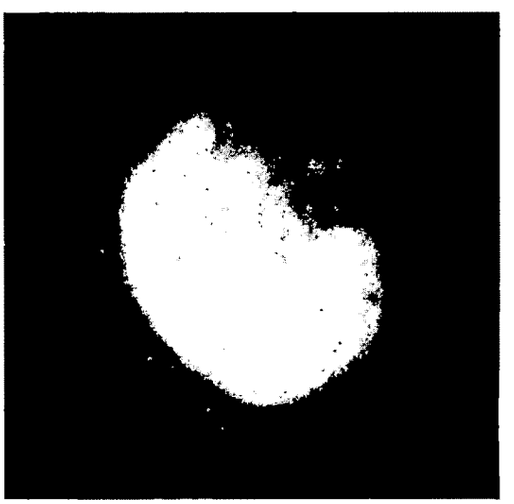


Fig. 1. Ultrasonographic diagnosis at 82 days' gestation, blighted ovum. Embryoscopy, abnormal residual embryo.

Knittlingen, Federal Republic of Germany).[2] The results were similar to the first series. I never observed complications such as rupture of the membrane, uterine perforation, or postoperative infection. However, I abandoned this endoscopic technique in viable pregnancies because of two main reasons: (1) at that time introduction of vaginal ultrasonography[3] convinced me that the same results of embryoscopy could be obtained by this noninvasive method in the first trimester of pregnancy, and (2) the extraembryonic cavities provide the surroundings in which the actual embryonic body starts its development and the consequences of chorion perforation are not known.

This personal experience leads me to disagree with the belief of Cullen et al. that direct access to the embryo by embryoscopy opens a new frontier into prenatal diagnosis and treatment, at least in viable pregnancies. However, embryoscopy could be applied as an investigative method in pathologic pregnancies (e.g., blighted ovum). I believe that in such cases our knowledge could be enhanced by the use of this technique (Fig. 1).

Giuseppe Ghirardini, MD
Division of Obstetrics and Gynecology, Franchini Hospital, 42027 Montecchio Emilia, RE, Italy

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Reply

To the Editors: Dr. Ghirardini states that we neglected to mention that previous authors had suggested that one could adequately visualize the embryo through the chorion. Our work clearly shows that one must penetrate the chorion to properly examine the embryo through the transparent amnion. Frankly, we did not think the above concept was worth debating.

We apologize for not referencing Dr. Ghirardini's experience, but we were not privy to information aired at the Naples conference in 1984. The other references he cited pertained to trophohlast sampling. In any case, we are delighted to hear about his experience with endoscopy.

We share Dr. Ghirardini's sentiments about the lack of potential for embryoscopy as a diagnostic tool for visualization of the embryo in continuing pregnancy.[1] However, we have found embryoscopy to be extremely useful in the validation of ultrasonographic findings before termination of pregnancy, evaluation of failed pregnancies, and we feel very strongly about its potential to gain access to the embryonic circulations for purposes of therapy.

Dr. Ghirardini's data would tend to support the safety and potential utility of this procedure.

Mark T. Cullen, MD
Department of Obstetrics and Gynecology, University of Florida, 655 W. 8th St., Jacksonville, FL 32209
John Whetham, MD, and John C. Hobbins, MD
Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT 06510

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Fetal-neonatal thrombocytopenia: Mode of delivery?

To the Editors: In their retrospective review of neonatal thrombocytopenia (Burrows RF, Kelton JG. Low fetal risks in pregnancies associated with idiopathic thrombocytopenic purpura. Am J Obstet Gynecol 1990;163:1147-50) Burrows and Kelton concluded that the relative lack of poor neonatal outcomes in cases of maternal idiopathic thrombocytopenic purpura prompted comment on obstetric management, including route of delivery. They suggest that because so many infants are unaffected by this disease that cesarean delivery cannot be justified in every case.

I have two problems with their publication. First, one must question their study group inasmuch as they did not require direct nor indirect antibody presence for a diagnosis of maternal disease. In fact, of the 46 mothers tested only eight had elevated platelet-associated immunoglobulin G. Is this the current standard in the field of hematology with regard to the diagnosis of idiopathic thrombocytopenic purpura? Second, the central question still remains unanswered: given a fetus with low platelet count as a result of maternally derived antibody, is abdominal delivery protective against fetal-neonatal hemorrhage?[1] Until this question is answered we will have to continue to rely on data such as published here, although it only indirectly touches on the basic problem.

Donald L. Levy, MD
Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, 825 Fairfax Ave., Norfolk, VA 23507

REFERENCE

1. Levy DL. Fetal-neonatal involvement in maternal autoimmune disease. Obstet Gynecol Surv 1982;37:122-7.

Reply

To the Editors: We thank Dr. Levy for his interest in our article. Dr. Levy raises two important points: the first is whether our patients had idiopathic thrombocytopenic purpura or whether they had a less severe illness, which would account for the good infant outcome. The second is whether the type of delivery should be an important consideration for infants of mothers with idiopathic thrombocytopenic purpura.

We are confident that our study group is representative of typical patients with idiopathic thrombocytopenic purpura. We were careful to exclude those patients with incidental thrombocytopenia of pregnancy because this condition does not carry risk for the mother or her fetus and by including such patients one would have an inaccurately optimistic picture of idiopathic thrombocytopenic purpura. Indeed, if anything, our patient population could be skewed toward a worst-case bias because we serve as a referral center for patients with idiopathic thrombocytopenic purpura. Idiopathic thrombocytopenic purpura is a diagnosis of exclusion and the measurement of platelet-associated immunoglobulin G is neither sufficiently sensitive nor specific to be used as a diagnostic test for the disorder. Furthermore, the likelihood of a positive result is directly related to the severity of the thrombocytopenia and consequently mothers with moderately severe thrombocytopenia who have ITP often have normal platelet-associated immunoglobulin G. If our patients are a true and representative example of typical pregnant patients with idiopathic thrombocytopenic purpura, what is the explanation for the relatively benign outcome our patients had compared with previous reports in the literature? We believe that the major factor is that our focus was on the fetal cord platelet count, the parameter of greatest interest to obstetricians when attempting to estimate the risk of bleeding in utero or at the time of delivery. Previous studies have focused on the platelet nadir, which we have shown typically occurs several days after delivery.

The real question is not whether abdominal delivery is protective but whether vaginal birth is harmful in infants of mothers having idiopathic thrombocytopenic purpura. Only when this risk is measured can the benefits of an intervention (cesarean section) be defined. Our contention is that, given the low risk of severe neonatal thrombocytopenia at the time of birth ($<20 \times 10^9$/L), we question whether routine cesarean section can ever be justified in pregnant patients with idiopathic thrombocytopenic purpura.

This is not to say that idiopathic thrombocytopenic purpura in pregnancy is not an important disorder; rather the greatest risk for the infant occurs several days after delivery and consequently the focus of concern shifts from the obstetrician to the pediatrician.

R.F. Burrows, MD, and J.G. Kelton, MD

Department of Obstetrics and Gynecology, McMaster University, 1200 Main St. West, Hamilton, Ontario, Canada L8N 3Z5

Parvovirus transmission concerns

To the Editors: The recent article by Rodis et al. (Rodis JF, Quinn DL, Gary GW Jr, et al. Management and outcomes of pregnancies complicated by human B19 parvovirus infection: a prospective study. Am J Obstet Gynecol 1990;163:1168-71) proposes a management protocol for evaluation of pregnant women exposed to B19 parvovirus in pregnancy. The initial step in this protocol is to determine the susceptibility of exposed women by performing serologic testing for the presence of B19-specific immunoglobulins. It is important to recognize that infected persons typically incubate the virus for approximately 1 week (usual range, 4 to 14 days) before becoming symptomatic and are likely to be contagious during this period without symptoms.[1] Occasionally, the contagious period may last as long as 20 days after exposure.[2] A helpful rule is that once the characteristic "slapped cheek" facial rash appears, there will be little or no virus remaining in respiratory secretions and therefore the person is extremely unlikely to be contagious. The incubation and transmission characteristics of B19 parvovirus raise concerns that inadvertent iatrogenic exposure to susceptible health care workers and obstetric patients may occur when an exposed patient is seen for serologic studies, genetic counseling, and ultrasonography. Caution must be exercised in these settings.

The Pregnancy Healthline at Pennsylvania Hospital has used a B19 parvovirus in pregnancy protocol that is remarkably similar to that used by Rodis et al. with the additional caution that serologic testing be performed at a facility that does not care for pregnant women or at a time that minimizes the risk of exposure of other pregnant women to a potentially infectious person. In addition, it is recommended that health care workers (physicians, sonographers, nurses, genetic counselors, phlebotomists, and clerical staff) who are likely to be exposed to these potentially infectious women should undergo serologic testing to determine their B19 parvovirus immunologic status.

B19 parvovirus may cause nonimmune hydrops, which can be successfully treated in utero.[3] This knowledge has led to pregnancy surveillance protocols that are designed to detect fetal compromise before deterioration is irreversible. In the course of using these protocols, it is important to avoid jeopardizing other pregnant women and minimize the further risk of transmission of B19 parvovirus.

Alan E. Donnenfeld, MD, and
Betsy Schick-Boschetto, RNC, MSN

Section of Genetics, Department of Obstetrics and Gynecology, Pennsylvania Hospital, Eighth and Spruce Streets, Philadelphia, PA 19107

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Reply

To the Editors: We appreciate the comments by Dr. Donnenfeld and Ms. Schick-Boschetto. We agree with them that iatrogenic parvovirus exposure of susceptible health care workers and obstetrics patients is to be avoided. We also agree that serologic testing for pregnant health care workers may be helpful so that those who are susceptible to infection can avoid exposure when possible, although there are no data to suggest that this is effective. Thus common sense should be used in screening patients for parvovirus infection just

as in any other potentially serious viral infection (e.g., varicella).

However, we recommend that caution be exercised when referring patients to commercial laboratories for parvovirus testing because during our outbreak both false-positive and false-negative results occurred when private laboratories were used. We suggest that whereas the blood sample may be obtained at any location, the specimen should be analyzed at a reference laboratory where quality control can be assured, such as the viral laboratory at the Centers for Disease Control or laboratories of the State Health Departments in the respective states.

John F. Rodis, MD
Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Connecticut Health Center, 263 Farmington Ave., Farmington, CT 06032

Computerized digital colposcopy

To the Editors: In a previous article, Crisp et al. emphasized the technology and possible applications of a computerized digital imaging colposcope (Crisp WE, Craine BL, Craine EA. The computerized digital imaging colposcope: Future directions. AM J OBSTET GYNECOL 1990;162:1491-8). For more than a year, we have had a positive experience with the same DENCOL 2100 computer system. Whereas standard colposcopy is a subjective diagnostic modality that depends on the experience and visual perception of individual colposcopists, computerized digital colposcopy has the capacity to objectively quantitate and analyze colposcopically visualized lesions. The system in its current form though has some limitations. The software needs to be refined. The menu requires some modifications, and color should be introduced to the black-and-white image to enhance the research and clinical potential of this promising technology.

The use of cervicography has been controversial.[1-3] Unlike cervicography, computerized digital colposcopy permits image processing and analysis, contrast and edge enhancement, computer-assisted quantification of lesions, and the detection of subtle changes. Colposcopic images can be improved with digital filters and stored. Image processing accentuates differences in cervical morphology and allows for the enhancement of capillary patterns, thereby emphasizing features of the cervix that might otherwise be missed. Serial measurements can be obtained to objectively evaluate progression-regression of cervical lesions. This computer-assisted quantitative-qualitative approach can lead to a more accurate and uniform appraisal of lesions visualized by colposcopy. It may improve our understanding of the natural history of cervical dysplasia and human papillomavirus infection of the genital tract.

Computerized digital colposcopy can be useful in the conservative management of patients with low-grade cervical dysplasia as well as in monitoring pregnant patients with abnormal cervical smears. Sequential computerized image analysis provides objective information as to whether a lesion is progressing, regressing, or stable in size. This may obviate the need for unnecessary active intervention in some cases. A study to evaluate the role of computerized digital colposcopy in the conservative management of low-grade cervical dysplasia is under way in our unit.

Magdy S. Mikhail, MD, and Seymour L. Romney, MD
Department of Obstetrics and Gynecology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461

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Reply

To the Editors: We are pleased to see comments by Drs. Mikhail and Romney that confirm many of the advantages we have previously discussed that can be realized by digital imaging colposcopy. With regard to improvements to the software, we note that Western Research Company, Inc. (Tucson, Ariz.), the originator of the digital imaging colposcopy system and software package, has continued to upgrade the software.[1] Changes suggested by Dr. Romney and others in private communications have been implemented and upgrades are available. This software package is continually evolving and Western Research Company anticipates offering a three-dimensional imaging version in the future.

We point out that this work has been supported by several research grants from the National Institutes of Health to one of us (B.L.C.). As a result, much of the development cost has been paid, allowing the provision to interested researchers or clinicians of a good digital imaging system for less than $6000, contrary to the figure mentioned in the comments on the article.

Comments with regard to color are of particular interest to us inasmuch as this is a very complex and poorly understood issue. Some of the adverse aspects of currently available color imaging systems for colposcopy have been addressed by Craine et al.[2] We have continued this research and have serious concerns about medical liability associated with some ill-conceived color systems, particularly as they relate to issues of spatial resolution and detection of high-frequency features in the resultant images. We have under way a research project for evaluation of specific color system parameters and will publish the results of that work when it is complete. In the meantime we will be happy to discuss the issues directly with interested parties.

We do see considerable potential for the use of color images in colposcopy, where the images have been obtained by use of specific color filters.[3] Again, however, we have determined that there are esoteric issues related to the convolution of detector response and broad-band optical filter characteristics, the understanding of which is critical to the limitation of liability. This liability potentially arises from the application of an incorrectly characterized system in which informa-

tion conveyed by the system is actually different from that the physician believes he is receiving. Publication on this subject is in preparation.

William Crisp, MD

Associated Gynecologists, Ltd., 1144 E. McDowell Road, Phoenix, AZ 85006

Brian L. Craine, MD, PhD

Western Research Company, Inc., 85 Bolinas Road, Suite 18, Fairfax, CA 94930

Eric R. Craine, PhD

Western Research Company, Inc., 2127 E. Speedway, Suite 209, Tucson, AZ 85719

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Questions about prophylactic intrapartum amnioinfusion

To the Editors: I read the article by Strong et al. (Strong TH Jr, Hetzler G, Sarno AP, Paul RH. Prophylactic intrapartum amnioinfusion: A randomized clinical trial. Am J Obstet Gynecol 1990;162:1370-5) with great interest and respect. Because we at Portsmouth Naval Hospital are still involved in an ongoing clinical trial to assess the efficacy of prophylactic amnioinfusion, I have several questions.

1. What ultrasonographic biometric measurements did you use to derive the estimated fetal birth weight? Was it done during labor? What was the mean percentage error of your estimated fetal weight? How many of the patients who were excluded solely on the basis of the estimated birth weight had birth weight >2500 gm? Was the birth weight of all the fetuses in the amnioinfusion and control groups >2500 gm? How would you manage a fetus with estimated birth weight <2500 gm and amniotic fluid index ≤5.0 cm?

2. Why warm the normal saline solution to 37° C before amnioinfusion? Miyazaki and Taylor[1] in the first known clinical trial of transcervical amnioinfusion stated that the "blood warmer . . . proved to be unnecessary as long as the infusion rate was limited to 10 to 15 ml per minute." Moreover, Miyazaki and Nevarez[2] in their randomized prospective study of amnioinfusion, had the "normal saline solution at room temperature." By advocating the use of normal saline solution warmed to 37° C aren't we adding an additional step that has not been shown to be necessary?

3. What was the mean (±SD) amniotic fluid index after amnioinfusion? Who performed the hourly amniotic fluid index—the same person or different people? Moore and Cayle have shown that the interobserver error for measurement of amniotic fluid index is 7%, whereas the intraobserver error is 3%. And if the amniotic fluid volume is <10 cm the error may be as high as 20%. Therefore it seems that to minimize error of hourly measurement of amniotic fluid index, the same person should take the measurements. Most important, do you recommend that an hourly amniotic fluid index be obtained during the first and second stage of labor if prophylactic amnioinfusion is used among patients with amniotic fluid index ≤5.0 cm?

4. How was fetal distress defined? Is it if there is no fetal heart rate acceleration with scalp or acoustic stimulation or if the scalp pH is <7.20? Was the same criterion of fetal distress used when performing emergency cesarean section as when doing forceps delivery? If not, then the conclusion that amnioinfusion significantly decreased "operative delivery for fetal distress" could probably more precisely read amnioinfusion significantly decreased the need to intervene with forceps for fetal distress. Were any of the authors involved with the decision to intervene because of fetal distress?

5. Although the umbilical arterial blood pH of the amnioinfusion group (7.29 ± 0.03) was "significantly higher than that of the control group (7.23 + 0.09)" what is the clinical significance of this observation? Isn't the critical question with umbilical arterial blood gases whether the neonate is acidotic, and if so, is it respiratory or metabolic acidosis?

6. Rutherford et al.[3] showed that the incidence of low 5-minute Apgar scores among fetuses with amniotic fluid index ≤5.0 cm was 11% but none of the 30 patients in the control group with amniotic fluid index ≤5.0 cm had a low Apgar score at 5 minutes. How do you account for this?

These questions by no means are supposed to distract from a well-designed and well-executed study.

Suneet P. Chauhan, MD

United States Naval Hospital, Naples, Italy, Box 19, FPO New York, NY 09521-0700

The opinions expressed in this letter are those of the author and not necessarily those of the United States Navy or the Department of Defense.

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3. Rutherford SE, Phelan JP, Smith CV, et al. The four-quadrant assessment of amniotic fluid volume: an adjunct to antepartum fetal heart rate testing. Obstet Gynecol 1987;70:353-6.

Reply

To the Editors: 1. The fetal head and abdominal circumferences were used to estimate fetal weights. All measurements were done in the latent phase of labor. None of the controls and only one in the study group had birth weights <2500 gm (2460 gm). Whereas no study has addressed prophylactic amnioinfusion for growth-retarded fetuses, in the setting of a reassuring fetal heart rate tracing, prophylactic amnioinfusion may not be unreasonable.

2. Our infusion rate was approximately 20 ml/min, thereby exceeding the "15 ml rule." When the am-

nioinfusate is held 4 feet above the infusion catheter tip, as it is during most amnioinfusion procedures, the rate of infusion will approach 20 ml/min.[1] Therefore many women who receive gravity-flow amnioinfusion may be at risk if room-temperature saline solution is used. Moreover, if amnioinfusion is reserved until variable decelerations occur, the fluid should be infused relatively rapidly. If the amnioinfusate is less than 3 feet above the catheter tip, the rate of amnioinfusion may be too slow to have utility.

3. The mean amniotic fluid index after amnioinfusion was 9.8 ± 2.9 cm (SD). As was stated in the article, only two investigators performed amniotic fluid index assessments. For any given subject, a single investigator performed all amniotic fluid index assessments. The majority (87%) who received prophylactic amnioinfusion maintained amniotic fluid index values above 8 cm for the duration of labor without requiring another amnioinfusion procedure. To reiterate our recommendations, hourly amniotic fluid index assessments after amnioinfusion are probably not necessary.

4. Fetal distress was generally defined as a decelerative fetal heart rate pattern with nonreactivity-decreased fetal heart rate variability that was not responsive to scalp or acoustic stimulation, or outright fetal bradycardia. The same criteria were used for both cesarean and forceps deliveries. As stipulated in the article, none of the authors was involved in the clinical management of any of the subjects.

5. Whereas there was a statistically significant difference in the pH values between the two groups, this difference does not appear to be clinically significant. However, as mentioned in the article, 30% in the noninfused group had pH values <7.20. Of these, 80% were consistent with metabolic acidosis.

6. The difference in the incidence of low 5-minute Apgar scores may be a reflection of the fact that the amniotic fluid index assessments performed by Rutherford were performed during the antepartum period, whereas those done in our study occurred during labor. Sarno et al.[2] assessed amniotic fluid index values early in the intrapartum period and found an incidence of low 5-minute Apgar scores, which was in agreement with that found in our control population.

Thomas H. Strong, Jr., MD
Phoenix Perinatal Associates, 1300 N. 12th St., No. 320, Phoenix, AZ 85006

REFERENCES

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2. Sarno AP, Ahn MO, Phelan JP. Intrapartum amniotic fluid volume at term: association of ruptured membranes, oligohydramnios and increased fetal risk. J Reprod Med 1990;35:719.

Panty hose–pants disease

To the Editors: Drs. Bergeron, Ferenczy, and Richart reported on the potential of self-contamination of HPV infections by underwear (Bergeron C, Ferenczy A, Richart R. Underwear: Contamination by human papillomavirus. AM J OBSTET GYNECOL 1990;162:25-9). At a recent lecture on the use of interferon in cases of genital papillomavirus (condyloma acuminatum), I commented that I had successfully used podophyllum resin, electrosurgery, cryosurgery, and autogenous vaccine. However, the most successful and simple treatment occurred about 7 years ago.

A patient in early pregnancy was seen with massive condylomata that encompassed the entire vulva and anal region. Simply, I told the patient to remove her panties and go without them, to wear only skirts or dresses during her entire pregnancy, and to insert one fourth applicator of sulfa cream vaginally every night. Much to my amazement the condylomata completely disappeared within 3 months. It remained clear throughout that pregnancy and delivery and a subsequent pregnancy. With only one case, I could not submit this for publication.

I believe that there is a panty hose–pants disease. Condyloma, *Monilia, Gardnerella,* and *Trichomonas* are involved. These diseases could have been instituted or aggravated with the advent of panty hose, jeans, sweat pants, and most recently leotards and aerobic suits. Recent published articles appear to concur. It is my opinion that we are seeing more of these infections because of the nonbreathable, tight nylon undergarments. I cannot recall seeing many of the mentioned infections in the late 1940s and early 1950s when loose panties (similar to boxer shorts) were commonly worn. It is conceivable that vulvovaginitis is not prevalent in those countries where women wear long dresses and loose undergarments.

Recently I have been advising those patients with recurring vulvovaginitis who insist on wearing panty hose to cut out the crotch or preferably wear loose cotton panties or no panties at all under a long dress. This would permit better air circulation to the anovulvar region, and has proved to be successful in minimizing recurrences.

This approach is old-fashioned common sense medicine to which I had been exposed, having obtained my MD degree in 1941 and completed my formal obstetrics and gynecology training in 1948. I hope that this might stimulate those in the research environment to do further research on the vulvovaginal infections.

Milton Turner, MD
900 E. 30 St., No. 202, Austin, TX 78705

Books received

A Clinical Infection in Obstetrics and Gynaecology. Edited by Allan B. MacLean. 392 pages. St. Louis, Missouri, 1990, Mosby–Year Book, Inc. $84.95.

Acupressure's Potent Points: A Guide to Self-Care for Common Ailments. Michael Reed Gach. 251 pages, illustrated. New York, 1990, Bantam Books. $14.95.

AIDS and Other Sexually Transmitted Diseases. Edited by Manuel Schydlower and Mary-Ann Shafer. 647 pages, illustrated. Philadelphia, 1990, Hanley & Belfus, Inc. $28.00.

Diagnosis and Treatment of Extrauterine Pregnancies. Nicholas Kadar. 256 pages. New York, 1990, Raven Press. $78.00.

Drugs in Pregnancy and Lactation: A Reference Guide to Fetal and Neonatal Risk. Third Edition. Gerald G. Briggs, Roger K. Freeman, and Sumner J. Yaffe. 732 pages. Baltimore, Maryland, 1990, Williams & Wilkins. $59.95.

The Family Practice Desk Reference. Second Edition. Charles E. Driscoll, Edward T. Bope, Charles W. Smith, Jr., and Barry L. Carter. 703 pages, illustrated. St. Louis, Missouri, 1990, Mosby–Year Book, Inc. $38.50.

The Family Practice Drug Handbook. Allan J. Ellsworth, Robert F. Bray, Brenda Sachse Bray, and John P. Geyman. 663 pages. St. Louis, Missouri, 1990, Mosby–Year Book, Inc. $21.95.

The Female Breast and Its Disorders. Edited by George W. Mitchell and Lawrence W. Bassett. 294 pages, illustrated. Baltimore, Maryland, 1990, Williams & Wilkins. No price listed.

F.I.G.O. Manual of Human Reproduction (3 Volumes). Volume I. Reproductive Physiology. Edited by Luigi Mastroianni and Christos Coutifaris. 114 pages, illustrated. **Volume II. Family Planning.** Edited by Mahmoud F. Fathalla, Allan Rosenfield, and Cynthia Indriso. 237 pages, illustrated. **Volume III. Reproductive Health.** Edited by Mahmoud F. Fathalla, Allan Rosenfield, Cynthia Indriso, Dilup K. Sen, and Shan S. Ratnam. 224 pages, illustrated. Park Ridge, New Jersey, 1989, The Parthenon Publishing Group, Inc. Three-volume set $90.00.

Healing the Infertile Family: Strengthening Your Relationship in the Search for Parenthood. Gay Becker. 310 pages. New York, 1990, Bantam Books. $12.95.

Human Prenatal Diagnosis. Second Edition. Edited by Karen Filkins and Joseph F. Russo. 624 pages, illustrated. New York, 1990, Marcel Dekker, Inc. $150.00.

Infectious Diseases. J. Schneider, J. Hughes, and A. Henderson. 490 pages. East Norwalk, Connecticut, 1990, Appleton & Lange. $70.00.

Modern Antenatal Care of the Fetus. Edited by Geoffrey Chamberlain. 436 pages, illustrated. St. Louis, Missouri, 1990, Mosby–Year Book, Inc. $104.95.

OB/GYN Notes. Roman T. Pachulski. 246 pages. Norwalk, Connecticut, 1990, Appleton & Lange. $16.95 (soft cover).

Obstetrics. Edited by Sir Alec Turnbull and Geoffrey Chamberlain. 1200 pages, illustrated. Edinburgh, 1989, Churchill Livingstone, Inc. $150.00.

Ovarian Cancer: Biological and Therapeutic Challenges. Edited by F. Sharp, W.P. Mason, and R.E. Leake. 481 pages, illustrated. New York, 1990, W.W. Norton & Company. $95.00.

Pathology of the Human Embryo and Previable Fetus. D.K. Kalousek, N. Fitch, and B.A. Paradice. 241 pages, illustrated. New York, New York, 1990, Springer-Verlag. $129.00.

Pregnancy, Autoimmunity, and Connective Tissue Disorders. J.S. Scott and H.A. Bird. 241 pages, illustrated. New York, New York, 1990, Oxford University Press. Price not listed.

Principles and Practice of Ultrasonography in Obstetrics and Gynecology. Fourth Edition. Arthur C. Fleischer, Roberto Romero, Frank A. Manning, Philippe Jeanty, and A. Everette James, Jr. 668 pages, illustrated. East Norwalk, Connecticut, 1990, Appleton & Lange. $95.00.

Progress in Obstetrics and Gynecology. Eighth Edition. John Studd. 399 pages, illustrated. New York, New York, 1990, Churchill Livingstone, Inc. $40.00.

Seasons of the Mind. Norman E. Rosenthal. 265 pages. New York, New York, 1990, Bantam Books. $9.95.

Syndromes of the Head and Neck. Third Edition. Robert J. Gorlin, M. Michael Cohen, Jr., and L. Stefan Levin. 977 pages, illustrated. New York, New York, 1990, Oxford University Press. Price not listed.

The Unborn Patient: Prenatal Diagnosis and Treatment. Second Edition. Edited by Michael R. Harrison, Mitchell S. Golbus, and Roy A. Filly. 626 pages, illustrated. Philadelphia, 1990, W.B. Saunders Company. Price not listed.

Unrecognized and Unsanctioned Grief: The Nature and Counseling of Unacknowledged Loss. Edited by V.R. Pine, O.S. Margolis, K. Doka, A.H. Kutscher, D.J. Schaefer, M. Siegel, and D.J. Cherico. 180 pages. Springfield, Illinois, 1990, Charles C Thomas, Publisher. $34.75.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Advances in Urogynecology, June 27-29, 1991, Hyatt Regency Hotel, Chicago, Illinois. For further information contact: Office of Continuing Medical Education, Northwestern University Medical School, 301 East Chicago Ave., Ward 1-004, Chicago, IL 60611-3008. Tel.: (312) 503-8533.

World Symposium of Perinatal Medicine, October 28-31, 1991, Sheraton Palace Hotel, San Francisco, California. For information contact: Contemporary Forums, 530 La Gonda Way, Suite E, Danville, CA 94526. Tel.: (415) 820-2800.

Thirteenth Course in Colposcopy and Cervico-Vaginal Histopathology (in English): Demonstration of the Use of Diathermy Loop, Paris, September 30–October 4, 1991. For information contact Dr. Cartier, 20 Rue des Cordelières, 75013 Paris, France. Tel.: 33.1.43.36.04.46.

Obstetric/Pelvic Imaging, Hands-On Instruction in Diagnostic Ultrasound. Conducted June 17-21, 1991, in Dallas, Texas. For further information call or write Keith Mauney & Associates, Inc., 600 East Las Colinas Blvd., Suite 250, Irving, TX 75039. Tel.: (214) 402-8250.

23rd Annual March of Dimes Clinical Genetics Conference, "Developmental and Genetic Disorders of the Central Nervous System," July 7-10, 1991, University of British Columbia, Vancouver, British Columbia, Canada. For further information contact Carol Blagowidow, March of Dimes. Tel.: (914) 997-4524.

Fetal Cardiology—Perinatal Cardiac Management, Malmo, Sweden, August 28, 1991, immediately prior to the 4th Congress of the International Perinatal Doppler Society, Program Directors James C. Huhta, MD, and Karl Marsal, MD. For information contact: Department of Ob/Gyn, Lund University, Malmo General Hospital, S-214-01 Malmo, Sweden. Tel.: 46-40-332-167; fax: 46-40-962-600.

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Stuart Weiner, M.D.
Section Chief,
Maternal-Fetal Medicine
Department of OB/GYN
Pennsylvania Hospital
8th & Spruce Streets
Philadelphia, PA 19107

Deadline for applications is **June 17, 1991.**

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Department of OB/GYN
S.U.N.Y., Health Science Center
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A full-time position is available in the Department of Obstetrics and Gynecology, University of California, Davis, Medical Center, Sacramento. Applicants should be Board eligible or certified in Obstetrics and Gynecology. Applicants must possess or be eligible for medical licensure in the State of California. Applicants must demonstrate experience and competence in teaching effectiveness and research related activities or potential for such activities. Special interest in the area of urogynecology, adolescent OB/GYN or sexual dysfunction is desired.

Responsibilities of this position include inpatient and outpatient case management, participation in teaching activities with residents, interns and medical students, development and/or participation in research activities and involvement in University and Hospital committees.

Send curriculum vitae and five references to:

R. Jeffrey Chang, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
1621 Alhambra Boulevard, Suite 2500
Sacramento, California 95816

The final date for receipt of applications is **June 1, 1991.**

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John W. Calkins, M.D., Interim Chairman
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Send C.V. to:

Mark Gibson, M.D.
c/o Marlene Hershman
Office of the Chairman
Department of Obstetrics and Gynecology
West Virginia School of Medicine
Health Sciences Center
Morgantown, West Virginia 26506

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University of Utah
Department of Obstetrics and Gynecology
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JULY, 1992

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Applicants should send a curriculum vitae to:

Method Duchon, M.D.
Acting Director, Division of
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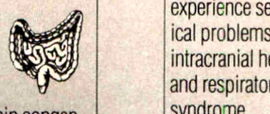
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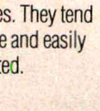
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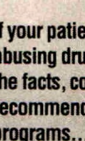


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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes; tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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‡Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents.

§An increase in blood pressure has been reported in women taking oral contraceptives.

1. Spellacy WN, Ellingson AB, Tsibris JCM. Glucose and insulin levels after six months of treatment with a triphasic oral contraceptive containing ethinyl estradiol and norethindrone. *J Reprod Med* 1989;34(8):540-542.

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ANTIMICROBIALS IN THE 1990s: EXPANDED OPTIONS FOR EFFECTIVE PATIENT CARE

Miami Beach, Florida
April 20-21, 1990

Guest Editor
Sebastian Faro, MD

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Oral ofloxacin was found to be as safe and effective as cefoxitin plus probenecid followed by doxycycline in the outpatient treatment of uncomplicated acute salpingitis.

The clinical experience with ofloxacin in the treatment of sexually transmitted diseases 1396
Michael L. Corrado, MD
Raritan, New Jersey

Sexually transmitted diseases caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis* cause significant morbidity in women. Ofloxacin is a recently introduced single-agent treatment for mixed infections.

Summary 1399
Sebastian Faro, MD, PhD
Houston, Texas

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Introduction

Sebastian Faro, MD, PhD

Houston, Texas

This symposium was held to present current data on the suitability of the quinolones in the treatment of infections in the gynecologic patient. The data from studies conducted at various hospitals from around the United States will serve to show that the quinolone, ofloxacin, is not only suitable but in some instances may be preferable to antibiotics currently in use.

Ofloxacin is unique in that it is active not only against *Neisseria gonorrhoeae* but also *Chlamydia trachomatis*. In addition, ofloxacin is also active against many gram-negative, facultative, anaerobic bacteria as well as gram-positive aerobes. Ofloxacin also has some activity against *Mycoplasma hominis* and *Ureaplasma urealyticum*. The fact that the quinolones, in general, do not have significant activity against the obligate anaerobic bacteria is of concern, especially when treating the patient with a soft tissue infection such as pelvic inflammatory disease.

The quinolones, especially ofloxacin, have been documented to be effective in the treatment of urinary tract infections, skin and soft tissue infections, and pulmonary infections. The data presented in these proceedings demonstrate that ofloxacin is effective in the treatment of female pelvic infections caused by *N. gonorrhoeae* and *C. trachomatis*. Initial studies have shown that ofloxacin is also effective in the treatment of pelvic inflammatory disease.

6/0/29045

Diagnosis and laparoscopic grading of acute salpingitis

David E. Soper, MD
Richmond, Virginia

Pelvic inflammatory disease continues to be a major cause of morbidity in women of reproductive age. Findings of bilateral adnexal tenderness and signs of a lower genital tract infection (mucopus, or leukorrhea, or both) should prompt clinicians to consider the diagnosis of salpingitis in this group of women. Additional signs of infection, such as elevated temperature, palpable adnexal complex, leukocytosis, elevated erythrocyte sedimentation rate, or c-reactive protein, and positive tests for either *Neisseria gonorrhoeae* or *Chlamydia trachomatis* will improve the overall specificity of the clinical diagnosis. Endometrial biopsy offers an acceptable approach to documenting objectively inflammation of the upper genital tract. Diagnostic laparoscopy should be considered in all patients but may be especially helpful for those patients in whom a diagnosis is unclear. A laparoscopic grading system based primarily on tubal mobility and inflammation can be useful in predicting duration of in-hospital therapy and future tubal factor infertility. (Am J Obstet Gynecol 1991;164:1370-6.)

Key words: Laparoscopic grading, pelvic inflammatory disease, obstetric and gynecologic infections, salpingitis

Pelvic inflammatory disease (PID) continues to be a major source of reproductive morbidity in young women.[1] Recent trends have revealed an increased rate and number of hospitalizations for PID as well as increases in ectopic pregnancy rates and infertility.[2-4] In addition, new information suggests that a large number of women suffer from atypical presentations of PID that are never clinically diagnosed or treated.[5,6] A high index of suspicion for the diagnosis of PID, especially in patients with atypical clinical presentations, in conjunction with appropriate clinical evaluation and judicious use of endometrial biopsy and diagnostic laparoscopy, may lead to an earlier diagnosis of PID and possibly decrease the incidence of sequelae.

Pathophysiology

Most PID in the United States is due to ascending infection by cervical microorganisms without prior surgical instrumentation. *Neisseria gonorrhoeae* is able to infect the endometrium and subsequently the fallopian tubes of approximately 15% to 30% of women who harbor this organism in their lower genital tract.[7,8] Cultures from the cervix have confirmed the association of *N. gonorrhoeae* with laparoscopically confirmed salpingitis in between 45% and 82% of cases.[9-12] Cultures from the fallopian tubes and peritoneum have confirmed the presence of *N. gonorrhoeae* in about half of those patients with lower tract infection.

Chlamydia trachomatis is difficult to culture from the upper genital tract, but direct fluorescent antibody stains and serologic studies suggest that the presence of this organism is responsible for as many as 65% of cases of salpingitis in some U.S. populations.[11,13] In addition, laparoscopic studies in Scandinavia have confirmed the presence of *C. trachomatis* in the fallopian tube in between 9% and 30% of patients with PID.[14-16]

Other microorganisms not believed to be sexually transmitted have also been implicated in the pathogenesis of salpingitis. Originally, culdocentesis studies suggested that as many as 80% of cases were associated with mixed infections of both aerobes and anaerobes.[17-19] However, a certain amount of contamination by resident vaginal flora occurred with this procedure.[17-21] North American laparoscopic studies confirm a polymicrobial origin of acute salpingitis in 8% to 40% of cases.[9-12] These microorganisms are particularly found in cases associated with tuboovarian abscesses. Whether these bacteria ascend from a source in the vagina or cervix or become translocated from the bowel lumen is unclear.[22] In unusual cases, appendicitis or Crohn disease can lead to a polymicrobial salpingitis.

This information on the bacterial pathogenesis of PID in North America is collected from only four studies,[9-12] consisting of 148 cases of laparoscopically confirmed salpingitis. Moreover, the data on chlamydia are predominantly based on findings in 48 patients all from

From the Department of Obstetrics and Gynecology and the Department of Internal Medicine, Division of Infectious Diseases, Medical College of Virginia, Virginia Commonwealth University.
Reprint requests: David E. Soper, MD, Box 34, Mer Station, Richmond, VA 23298.
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the same medical center in Seattle.[11, 13] In addition, a substantial number of laparoscopically confirmed cases have negative culture findings of the fallopian tubes and peritoneal fluid. Thus our understanding of the microbial cause of PID is far from complete.

Diagnosis

The patient must come for evaluation for the physician to make a clinical diagnosis of PID. The chief complaint of most patients diagnosed with PID is lower abdominal pain. However, as evidence mounts supporting the existence of atypical salpingitis, other symptoms referable to the genitourinary tract should be considered as potential indicators of upper genital tract infection. These symptoms include dyspareunia, dysmenorrhea, menorrhagia, metrorrhagia, and vaginal discharge.[5] Urinary tract symptoms, such as dysuria and frequency, may also be indicative of infection with a sexually transmitted pathogen.

Infection of the lower genital tract with either *N. gonorrhoeae* or *C. trachomatis* increases a patient's risk for upper tract infection. Risk factors that promote infection with these microorganisms include age younger than 25 years and multiple sexual partners. Patients with bacterial vaginosis and those who frequently douche may also have an increased risk of PID.[23, 24] Iatrogenic procedures, such as intrauterine device insertions and dilation and curettage, which breech the cervical mucous barrier and place potentially pathogenic microorganisms into the endometrium, can also lead to upper tract infection.

Other signs that may herald the onset of PID are breakthrough bleeding on the birth control pill or persistent vaginal spotting after elective termination of pregnancy. These complaints commonly result from an endometritis, which, if not treated, may progress to salpingitis.

Physical findings consistent with a diagnosis of PID include the presence of cervical mucopus, leukorrhea, or both.[25, 26] After cleansing the ectocervix with a large cotton swab, a cotton swab should be placed into the endocervical canal and inspected for a green or yellow color. In addition, Gram's stain of this material after it is streaked on a slide should be examined microscopically for the presence of white blood cells. More than 30 polymorphonuclear cells per oil immersion field correlates with mucopurulent cervicitis and chlamydial or gonococcal infection.[27] In addition, microscopy of a wet mount of the vaginal secretions should reveal a ratio of more than one white blood cell per epithelial cell in patients with PID. In a patient with lower abdominal pain, the presence of bilateral adnexal tenderness together with signs of a lower genital tract infection (mucopus, leukorrhea, or both) is associated with the laparoscopically confirmed diagnosis of acute salpingitis in 65% of cases.[28]

Additional clinical findings supportive of infection and inflammation improve the specificity of the clinical diagnosis of PID. Elevated temperature, palpation of an adnexal complex, leukocytosis, elevated erythrocyte sedimentation rate or C-reactive protein, purulent material obtained by culdocentesis, or a positive test for a lower genital tract infection with *N. gonorrhoeae* or *C. trachomatis* are considered adjunctive criteria for the diagnosis of PID. The specificity of the clinical diagnosis of PID increases to greater than 90% when two or more of these criteria are associated with the findings of pain, adnexal tenderness, and leukorrhea.[29] The take-home message is clear. Although diagnostic laparoscopy is useful in obtaining reliable cultures and assessing the severity of disease in overt cases of salpingitis, it is most helpful in confirming the diagnosis of salpingitis in the patient with a mild clinical presentation.

Endometrial biopsy and culdocentesis can also be used to confirm the presence of inflammation of the upper genital tract. In comparison with laparoscopically confirmed salpingitis, endometritis had a positive predictive value of 84% in the diagnosis of PID.[30] Moreover, when five or more neutrophils per ×400 field in endometrial surface epithelium are associated with one or more plasma cells per ×120 field in the endometrial stroma, a sensitivity of 92% and a specificity of 87% for predicting the diagnosis of both laparoscopically confirmed acute salpingitis and a positive upper genital tract culture are noted.[31] Most recently, endometrial biopsy was more than 90% specific in predicting histopathologic evidence of salpingitis.[32] Culdocentesis showing purulent material has also been suggested as a supportive criterion for the diagnosis of PID.[33] This finding in conjunction with the clinical signs and symptoms of PID should have a high positive predictive value if an adequate specimen can be obtained. Unfortunately, only 60% of patients with a clinical diagnosis of PID will have a positive test result because of technical difficulties, absence of cul-de-sac pus, or possibly incorrect diagnosis.[34] Culdocentesis is associated with significant patient discomfort and for this reason does not enjoy widespread use for the diagnosis of either PID or ectopic pregnancy. On the other hand, endometrial biopsy is highly recommended in the workup of a patient suspected of having PID. The procedure is technically uncomplicated and can be performed by both primary care physicians and gynecologists. It allows the clinician to partially evalute the upper genital tract but stops short of requiring general anesthesia and laparoscopy. When performed with the Pipelle endometrial suction curette, only minor discomfort is experienced by the patient.

Table I. Clinical criteria for the diagnosis of PID

Westrom and Mardh[38]	*Hager*[33]	*Suggested*
Major criteria (all must be present)		
Abdominal pain	Abdominal tenderness	Adnexal tenderness
Adnexal tenderness	Cervical motion tenderness	Signs of a LGTI
Signs of a LGTI	Adnexal tenderness	
Minor criteria (additional criteria increase the specificity of the diagnosis)		
Palpable adnexal mass	Gram's stain of endocervix—positive for GND	Endometrial biopsy = endometritis
ESR ≥ 15 mm/hr	Temperature > 38° C	Elevated CRP or ESR
Temperature > 38° C	Leukocytosis (> 10,000)	Temperature > 38° C
	Purulent material by culdocentesis	Leukocytosis
	Pelvic complex by examination or sonography	Positive test for chlamydia or GC

LTGI, Lower genital tract infection; *ESR*, erythrocyte sedimentation rate; *GND*, gram-negative diplococci; *CRP*, C-reactive protein; *GC*, *N. gonorrhoeae*.

The clinical criteria for the diagnosis of PID have never been validated in large prospective studies. Currently two similar sets of guidelines for the diagnosis of PID have been suggested. Jacobson and Westrom were[28] the first to evaluate the accuracy of conventional signs and symptoms of PID as assessed by the visual confirmation of acute salpingitis. Diagnostic accuracy of acute salpingitis was improved by increasing the number of positive parameters. Westrom suggested that the basis for the diagnosis of PID should be a minimum of three major criteria, with supportive signs, laboratory tests, or both improving the specificity of the diagnosis (Table I). Of particular importance is Westrom's uniform finding of a marked increase in the number of inflammatory cells in the wet smear of the vaginal secretions of women with PID.[25] It appears that this simple test can be used to exclude the possibility of PID in women with abdominal pain.

In a separate analysis of Westrom's data, purulent vaginal discharge, an elevated erythrocyte sedimentation rate, positive culture for *N. gonorrhoeae*, adnexal swelling on bimanual examination, and a temperature greater than or equal to 38° C were good predictors of laparoscopically confirmed salpingitis.[29] Hager et al.[33] published similar guidelines suggesting that clinicians rely on three major clinical signs and at least one of five supportive parameters. Their guidelines eliminate the necessity for a chief complaint of abdominal pain, an important improvement if we accept the probability that many patients with atypical ("silent") salpingitis may not have abdominal pain.[5,6] However, they diminish the importance of abnormal vaginal secretions, although a Gram's stain of the endocervix revealing gram-negative diplococci is an adjunctive criterion (Table I). As Hager et al.[33] point out, it is important to use criteria that will not exclude patients with mild disease. A compromise between the two published guidelines should allow the desired high sensitivity with respect to the diagnosis of PID. Patients who come to the clinician with any symptoms referable to the genitourinary tract, have bilateral adnexal tenderness during bimanual pelvic examination, and have signs of an infection of the lower genital tract, specifically microscopy of a wet mount of the vaginal secretions showing increased numbers of inflammatory cells, should have a diagnosis of PID considered. Endometrial biopsy should also be considered to confirm the clinician's suspicion of upper genital tract inflammation. Further study and validation of clinical diagnostic criteria should be undertaken, but until that is accomplished, this approach will allow potential morbidity from atypical salpingitis to be minimized. This will inevitably lead to the treatment of patients for PID who in reality do not have salpingitis. However, many of these patients will have endocervicitis or endometritis without salpingitis, and therefore antibiotic treatment is indicated in any case. Because of the expected low specificity of the diagnosis of PID with respect to confirmed salpingitis in these cases, rigorous scientific study of this disease will continue to require laparoscopy as the gold standard for the diagnosis and evaluation of the severity of disease.

The clinical grading[33] or staging[35] of salpingitis may be helpful in clinical triage regarding whether a patient should be admitted to the hospital, but its relationship to laparoscopic findings has not been determined. At the Medical College of Virginia, patients admitted with four-quadrant rebound and significant elevation in temperature are more likely to have a laparoscopically mild salpingitis (tubes freely mobile and ostia appear patent) rather than a ruptured tuboovarian abscess (unpublished observations). Moreover, bimanual pelvic examination in patients with significant direct and indirect tenderness can rarely be performed sufficiently to determine if the patient has a palpable adnexal complex, which leads to incorrect clinical staging in many patients. It is clear that the clinical severity of disease

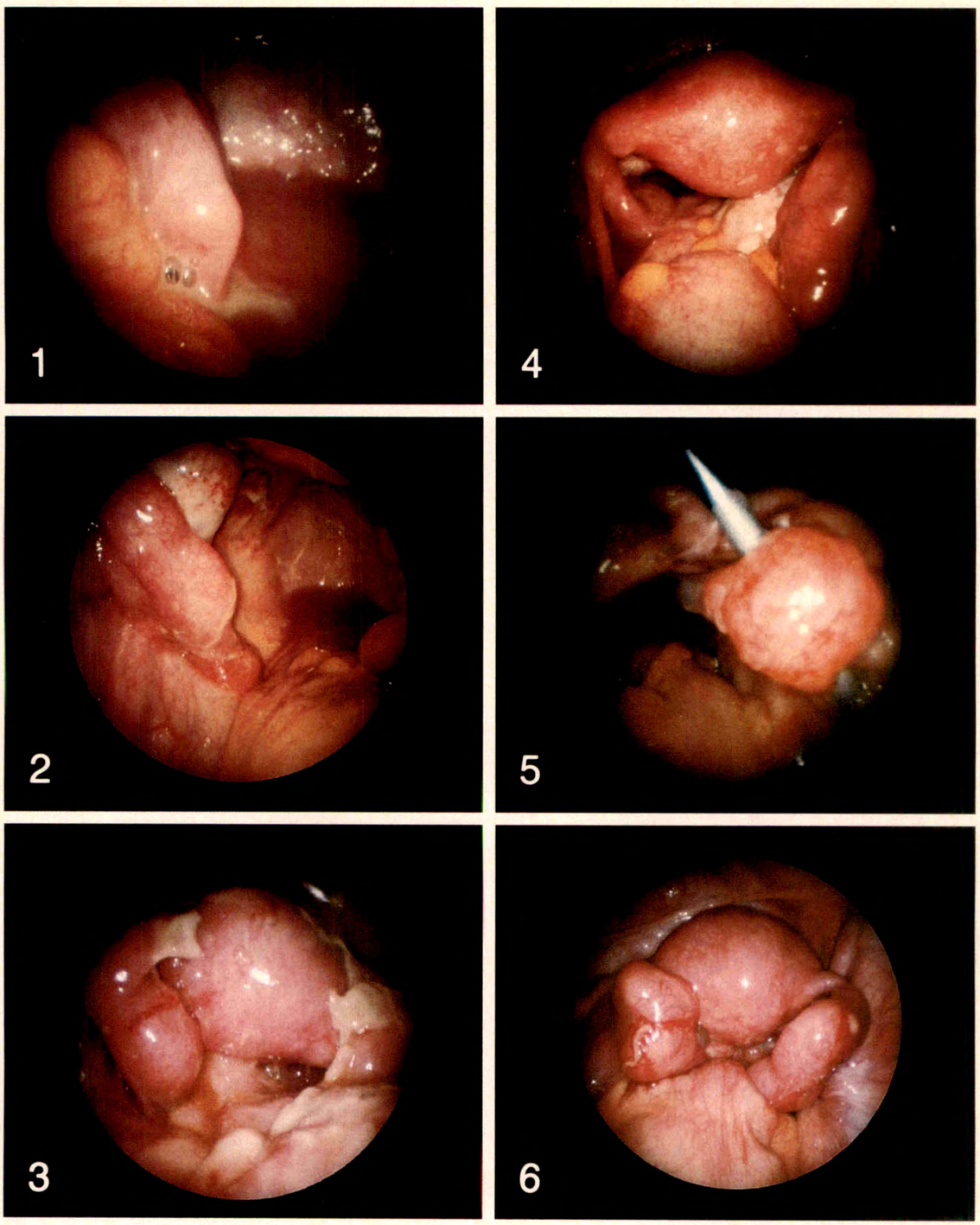


Fig. 1. A freely mobile, patent fallopian tube with purulent material exuding from the ostia, is seen resting in a pool of serosanguinous, purulent exudate. **Fig. 2.** The left fallopian tube is covered with a sticky exudate. A serosanguinous, purulent exudate is seen in the cul-de-sac. **Fig. 3.** Patchy fibrin deposits are seen on the serosal surfaces of the tubes. **Fig. 4.** The left fallopian tube is adherent to the posterior aspect of the broad ligament and an adhesion can be seen extending from the tube to the bowel. **Fig. 5.** Fimbria of the right fallopian tube are agglutinated and swollen in a patient with moderate salpingitis. **Fig. 6.** Bilateral pyosalpinx are noted. Puncture wounds from an 18-gauge spinal needle are noted to exude purulent material.

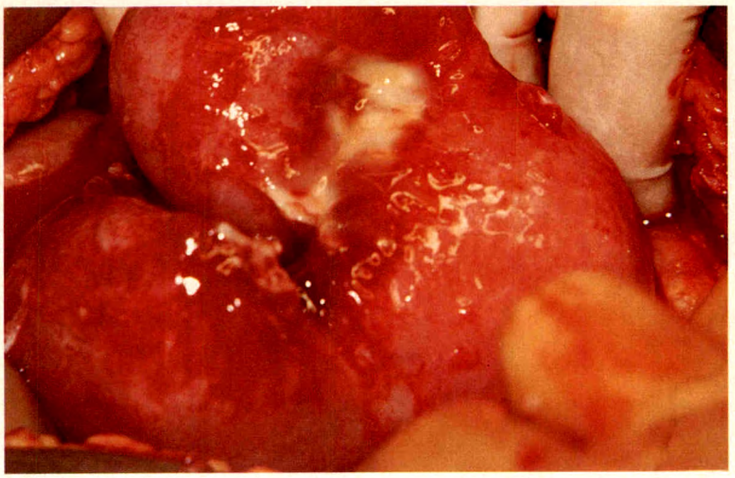


Fig. 7. A left tuboovarian abscess is lifted out of an incision. The uterus is seen anteriorly. This patient required a total abdominal hysterectomy–bilateral salpingo-oophorectomy for failure to respond to antibiotic therapy.

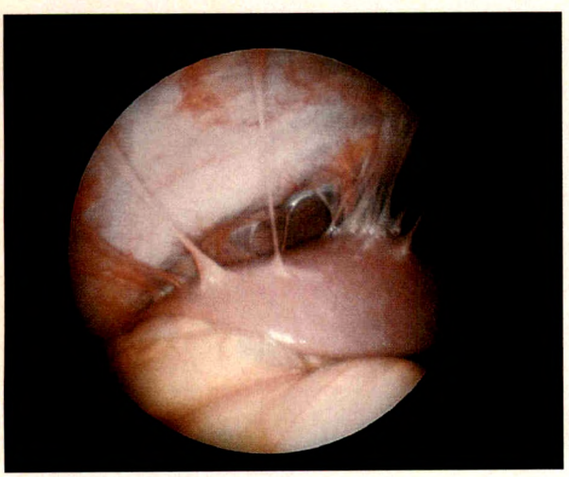


Fig. 8. Violin-string adhesions are visualized in this patient with Fitz-Hugh–Curtis syndrome.

as assessed by physical examination has no relationship to the grade of laparoscopically confirmed salpingitis with respect to tubal mobility and patency.[36]

Laparoscopic grading

The minimum criteria for the visual confirmation of acute salpingitis include (1) pronounced hyperemia of the tubal surface, (2) edema of the tubal wall, and (3) a sticky exudate on the tubal surface and from the fimbriated ends when patent.[28] In some cases erythema and edema can be overinterpreted because of observer bias.[32] For this reason, visual confirmation of salpingitis without the presence of exudate may be erroneous (false positive).[30] Evaluation of the peritoneal exudate to document the presence of inflammatory cells is suggested. Peritoneal fluid in normal patients and in those with endometriosis will contain predominantly macrophages.[37] Peritoneal fluid cytologic examinations in patients with acute salpingitis will inevitably reveal a predominance of neutrophils.[30] The value of histologic evaluation of minute fimbria biopsy specimens to confirm pathologically the visual diagnosis of salpingitis has yet to be defined. However, Sellors et al.[32] have demonstrated the importance of minute fimbria biopsy as an additional objective parameter in preventing both a false-positive and false-negative laparoscopic diagnosis of salpingitis. Most important, patients who do not meet the minimum criteria for visual confirmation of salpingitis may actually have subclinical disease, possibly because of an endosalpingitis. Minute fimbria biopsy may play a role in defining this subset of women with visually mild salpingitis. Moreover, it is imperative to perform an endometrial biopsy during the performance of the diagnostic laparoscopy to add objective evidence of upper genital tract inflammation.

Criteria for the severity of salpingitis by laparoscopic examination have been reported by Westrom.[38] These criteria do not differ significantly from the refined criteria of Hager et al.[33] Mild salpingitis is associated with the above minimum visual criteria. In addition, the tubes are freely mobile and the ostia appear patent (Fig. 1). The tube may be covered with a stickly exudate (Fig. 2). Moderate salpingitis is associated with more pronounced inflammation. Patchy fibrin deposits are on the serosal surfaces and the tubes are not freely movable (Fig. 3). Adhesions are loose and moist, and a paraphimotic appearance may be present (Fig. 4). The fimbria may appear adherent (Fig. 5). Severe sal-

pingitis reveals intensely congested peritoneal surfaces. Pelvic organs adhere to each other, and pyosalpinx (Fig. 6) or tuboovarian complex formation (Fig. 7) may be present.

In some cases both adnexa are not equally affected by the inflammatory process. Grading should be performed with respect to each individual adnexal finding. The overall grade should reflect the most severe finding. For example, a patient with a right-sided pyosalpinx but an otherwise mildly involved left tube would warrant an overall grade of severe. The severity of the grade of salpingitis is, for the most part, dependent on tubal mobility and patency. Therefore an acute infectious process superimposed on chronic adhesive changes, possibly because of a previous episode of salpingitis, will be graded as more advanced when in reality the amount of purulent exudate, side-to-side agglutination, and tubal edema and erythema may be less than an initial mild grade of gonococcal salpingitis. Despite this possibility, patients with a more severe grade of salpingitis require a longer duration of antibiotic therapy before reaching a therapeutic response.[11] As previously mentioned, the clinical severity of disease does not predict the severity of tubal disease. In fact those patients with milder disease, as seen in patients with chlamydial salpingitis, actually have more severe inflammatory changes in the tubes than would be expected from a relatively benign clinical course.[31]

Sequelae

The sequelae resulting from salpingitis is directly proportional to the severity of the inflammatory reactions of the tubes. The incidence of infertility caused by a single episode of severe salpingitis appears to be close to 30% and falls to a low of 6% in patients with only mild disease.[38] Patients taking oral contraceptives at the time of their diagnosis appear to have an improved prognosis, not only are they less likely to develop salpingitis initially, but they generally have a less severe grade of salpingitis if they do develop upper tract disease.[39] Rates of tubal factor infertility also increase with subsequent episodes of PID. The patient with three or more episodes of salpingitis runs a 50% to 60% risk of tubal factor infertility.[38] Despite earlier data to the contrary, a prognosis of fertility does not appear to be related to the microbial cause of acute salpingitis.[40]

The Fitz-Hugh–Curtis syndrome is characterized by violin-string adhesions between the liver and anterior abdominal wall in women with gross pathologic evidence of prior tubal infection. It consists of a continuum of acute perihepatitis associated with acute salpingitis followed by the formation of perihepatic adhesions (Fig. 8). Occasionally chronic right upper quadrant pain necessitating laparoscopic lysis of adhesions occurs.[41]

Summary

Although much has been learned about the pathophysiology and diagnosis of PID during the past decade, much more remains to be investigated. Unified clinical diagnostic criteria must be developed and validated in large prospective studies. Less invasive methods for confirming the diagnosis and assessing the severity of tubal disease also must be evaluated. Most important, further clarification of the pathophysiology and clinical presentation of atypical or "silent" salpingitis must be elucidated. Only in these ways can strategies for the prevention and intervention be developed to decrease the serious sequelae associated with PID.

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Patient costs for prophylaxis and treatment of obstetric and gynecologic surgical infections

Gary E. Stein, PharmD
East Lansing, Michigan

The use of newer broad-spectrum antimicrobials for prophylaxis and treatment in obstetric and gynecologic surgery can reduce patient morbidity and hospital costs. For prophylaxis, a single dose of a cephalosporin with a long elimination half-life can be as effective as a more prolonged course. Single-dose prophylaxis reduces not only toxicity and cost to the patient but also the likelihood of colonization of the vagina by resistant organisms. Treatment regimens for postoperative pelvic infections should have broad-spectrum coverage against aerobic and anaerobic pathogens to ensure high cure rates and prevent subsequent abscess formation. With the introduction of newer cephalosporins and penicillin combinations that include a β-lactamase inhibitor, it is now possible to treat these polymicrobial infections effectively with monotherapy. Compared with traditional antibiotic combinations, these drugs can reduce side effects and the costs of drug administration as well as the need for therapeutic monitoring. The use of oral antibiotics to complete a course of treatment can also help decrease the high costs of parenteral antibiotic therapy and hospitalization. (AM J OBSTET GYNECOL 1991;164:1377-80.)

Key words: Costs, prophylaxis, obstetric and gynecologic surgical infections, quinolones

Infection after obstetric and gynecologic surgery continues to be an important source of patient morbidity despite improved surgical techniques and the use of prophylactic antibiotics. The cause of pelvic infections after common procedures such as vaginal hysterectomy or cesarean section has now been better defined and often involves both aerobic and anaerobic bacteria.[1] In addition to well-recognized pathogens such as β-hemolytic streptococci, *Escherichia coli, Neisseria gonorrhoeae, Chlamydia trachomatis,* and coagulase-negative staphylococci, anaerobic bacteria are commonly recovered in patients with postoperative endomyometritis or pelvic cellulitis.[2] The most common anaerobic organisms isolated include peptostreptococci, peptococci, *Bacteroides bivius, B. disiens, B. fragilis,* and *Fusobacterium* species. Because of the polymicrobial nature of these infections, it is important to use treatment regimens that have a broad spectrum of activity that includes aerobic and anaerobic bacteria.

Prophylaxis

Antibiotics given before obstetric and gynecologic surgery can significantly decrease the number of operative site infections, reduce febrile morbidity, and shorten hospital stay.[3] The overall effectiveness of antibiotic prophylaxis is influenced by several factors, which include surgical technique, duration of the surgery, and the antibiotic chosen. It is vitally important that the antibiotic has not only good activity against potential pathogens but also attains high tissue levels at the time of bacterial contamination. Shapiro et al.[4] found that the salutary effect of prophylactic cefazolin diminished rapidly with increased duration of surgery.

The ideal prophylactic antibiotic should be nontoxic, effective against most organisms encountered in the endogenous flora, long acting, and inexpensive. Although numerous antibiotics have been found to be effective prophylactic agents, cefazolin continues to be the recommended choice in gynecologic surgery.[5] This cephalosporin has been studied extensively and has the advantage of a long half-life. A cost-benefit analysis of a three-dose regimen of cefazolin for prophylaxis found an average benefit of $492 in vaginal hysterectomy and a $102 savings in patients undergoing abdominal hysterectomy.[6] Longer durations of prophylaxis or the use of more expensive cephalosporins were found to diminish these cost benefits. Zhanel et al.[7] calculated that approximately $25,000 could be saved from their annual pharmacy budget if cefazolin was substituted for more expensive antimicrobials for prophylaxis in obstetric and gynecologic surgery.

Because anaerobes such as *B. fragilis* are frequently recovered in postoperative pelvic infections, the need for prophylactic antibiotics with activity against these

From the Department of Medicine, Michigan State University School of Medicine.
Reprint requests: Gary E. Stein, PharmD, Department of Medicine, Michigan State University, 13-220 Life Sciences Building, East Lansing, MI 48824.
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organisms has been advocated by some investigators. Hamond et al.[8] demonstrated equal effectiveness of cephalothin and metronidazole in preventing infection after vaginal hysterectomy. This suggests that prophylactic agents need not be effective against all possible pathogens, but activity against either aerobic or anaerobic flora would reduce postoperative infections. Other investigations have shown that antibiotics that are active against both aerobic and anaerobic organisms were more effective than agents with just an aerobic or an anaerobic spectrum. This has led to studies with broad-spectrum antibiotics such as cefoxitin, piperacillin, and more recently cefotetan. These antibiotics are active against aerobic and anaerobic pathogens associated with pelvic infections and have been found to be effective prophylactic agents. In addition, Stiver et al.[9] observed a lower fever index and shorter hospital stays in patients in whom infections developed after cesarean section when they received prophylactic cefoxitin compared with cefazolin. Cefotetan has an advantage over these other antibiotics because of its extended half-life. A single, perioperative dose of cefotetan has been found to be as safe and effective as a three-dose regimen of cefoxitin for prophylaxis in cesarean section and hysterectomy.[10, 11]

Antibiotic prophylaxis has become standard care for obstetric and gynecologic operations, but the most appropriate agent and duration of antibiotic administration have not been defined precisely. It appears that a single dose of an antibiotic with a long half-life can be as effective as prolonged courses of prophylaxis. A single, preoperative dose has the potential advantages of reduced cost and toxicity to the patient as well as reduced likelihood of increased colonization of the vagina by resistant organisms.

Treatment

With the recognition that postoperative pelvic infections are frequently polymicrobial in nature, newer treatment strategies have recommended antibiotic regimens with activity against aerobic and anaerobic pathogens.[12] The rationale for these recommendations has been formulated from animal models such as the intraabdominal sepsis model of Weinstein et al.[13] These investigators found that untreated peritonitis resulted in a high mortality rate that was caused by gram-negative facultative bacteria such as *E. coli*. Abscesses observed in surviving animals appeared to be caused by microbial synergy between facultative and anaerobic bacteria. Only antibiotic regimens with both aerobic and anaerobic coverage were able to decrease mortality and the subsequent development of abscesses.[14] Although treatment of these polymicrobial infections has traditionally been with a combination of an aminoglycoside plus clindamycin, single broad-spectrum antibiotics are also effective. Monotherapy helps avoid side effects as well as the increased indirect costs of drug administration and therapeutic monitoring.

Several newer antimicrobials have been suggested as alternatives to combination therapy. These include cephalosporins (cefoxitin and ceftizoxime), extended-spectrum penicillins (mezlocillin and piperacillin), monobactams (aztreonam), carbapenems (imipenem), and fluoroquinolones (ofloxacin and ciprofloxacin).[15] These antibiotics are generally safe and effective alternatives to aminoglycosides, but several lack adequate anaerobic coverage and still require the addition of clindamycin or metronidazole in the treatment of mixed anaerobic-aerobic infections. In addition, some of these drugs are not suitable for monotherapy because resistance or superinfection commonly develops during therapy. Numerous clinical trials have demonstrated the effectiveness of cefoxitin, and this antibiotic has generally been the preferred single agent for therapy of postoperative pelvic infections during the past decade.[16] Recently newer cephalosporins such as ceftizoxime and cefotetan have been found to be effective in the treatment of acute obstetric and gynecologic infections.[17] Because these antibiotics require fewer daily doses, they have become cost-effective alternatives to cefoxitin. Sochalski et al.[18] found that the total cost per day of cefotetan was approximately 40% lower than cefoxitin in their hospital. One potential drawback to the use of these newer cephalosporins is their higher rates of resistance against clinically important anaerobic bacteria (Table I).[19]

Of the newer broad-spectrum antimicrobials that have been introduced during the past few years, the single antibiotic combinations may prove to be the most useful for empiric treatment of polymicrobial infections. Combinations of older penicillins, such as ampicillin and ticarcillin with a β-lactamase inhibitor, are active against a large number of facultative bacteria. In addition, they are essentially always active against anaerobes and need not be tested for clinical purposes.[20] Clinical evaluations of ticarcillin-clavulanate and ampicillin-sulbactam have shown that they are as effective as cefoxitin, as well as combination therapy, in the treatment of pelvic infections.[21, 22] These single antibiotic combinations need to be administered more frequently than some of the newer cephalosporins, which makes them more costly to administer in the hospital. On the other hand, fewer treatment failures may occur with these drugs because of their expanded spectrum.[23] Imipenem-cilastatin is another single antibiotic combination that has been effective in the treatment of obstetric and gynecologic infections.[24] Unlike sulbactam and clavulanate, cilastatin does not inhibit bacterial β-lactamases but prevents renal enzymes from inactivating imipenem. Imipenem has the broadest coverage of

Table I. Activity of antimicrobial agents against *B. fragilis* species

Drug	Breakpoint (μg/ml)	% of species susceptible at the breakpoint	
		B. fragilis sp	*B. fragilis group**
Imipenem	8	100	100
Ampicillin/sulbactam	16	100	100
Metronidazole	16	100	100
Clindamycin	4	93	81
Cefoxitin	32	92	75
Piperacillin	128	84	85
Ceftizoxime	32	43	45
Cefotetan	32	85	56

From Finegold SM, Wexler HM. Antimicrob Agents Chemother 1988;32:611-6, by permission of American Society for Microbiology.

*Includes *B. fragilis* species.

Table II. Patient costs for monotherapy of postoperative pelvic infections

Drug	Dose/schedule	Cost per day*
Cefotetan	2 gm every 12 hr	$ 62
Ampicillin/sulbactam	3 gm every 6 hr	$ 84
Ticarcillin-clavulanate	3.1 gm every 6 hr	$ 84
Ceftizoxime	2 gm every 8 hr	$ 93
Cefoxitin	2 gm every 6 hr	$108
Imipenem	1 gm every 8 hr	$144

*Average wholesale cost + administrative fee ($10).

any single antibiotic, but because of its high cost it should be reserved for resistant cases (Table II).

Because of the high cost of parenteral therapy and hospitalization, oral antibiotics should be considered to complete a course of therapy. The single antibiotic combination of amoxicillin-clavulanate is the oral equivalent of ampicillin-sulbactam and is useful in the treatment of polymicrobial infections.[25] Other antibiotic combinations, such as a fluoroquinolone plus metronidazole, can also be effective oral therapy. The fluoroquinolones are broad-spectrum antibiotics and attain high concentrations in reproductive tissues.[26] Ofloxacin may be especially useful in some patients because of its increased activity against *Chlamydia* and *Mycoplasma*.[27-29]

Conclusion

A large body of knowledge now exists concerning appropriate prophylaxis and treatment of obstetric and gynecologic infections. Effective prophylaxis can be accomplished with a single dose of a long-acting cephalosporin, such as cefazolin or cefotetan. Empiric treatment of postoperative pelvic infections requires an antimicrobial regimen with coverage against aerobic and anaerobic organisms. Monotherapy with a broad-spectrum cephalosporin or a penicillin combination that includes a β-lactamase inhibitor has been found to be as effective as combination chemotherapy. The conversion to oral antibiotics, when appropriate, will also help decrease treatment costs and hospital stay.

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Effectiveness of ofloxacin in the treatment of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* cervical infection

Sebastian Faro, MD, PhD, Mark G. Martens, MD, Maurizio Maccato, MD, Hunter A. Hammill, MD, Scott Roberts, MD, and Gerald Riddle, MS

Houston, Texas

Forty patients with cervical infection caused by *Chlamydia trachomatis* were treated with ofloxacin (20) or doxycycline (20). Ofloxacin was successful in eradicating *C. trachomatis* from all 20 (100%) patients. Doxycycline was effective in 18 of 20 (90%) patients. Three patients had a concomitant cervical gonococcal infection. All three were successfully treated: one with ofloxacin and two with doxycycline. Ofloxacin, 300 mg, taken twice daily for 7 days, is effective in eradicating endocervical *C. trachomatis* infection. (Am J Obstet Gynecol 1991;164:1380-3.)

Key words: Cervicitis, *C. trachomatis, N. gonorrhoeae*

Antibiotic therapy for the treatment of cervicitis caused by *Neisseria gonorrhoeae* is predicated on the suspicion that a penicillinase-producing strain may be present. In addition, *N. gonorrhoeae* is known to have both plasmid and chromosomal resistance, thus making penicillin, tetracycline, and cefoxitin less effective.[1] In a recent study of 6204 gonococcal isolates from 21 locations within the United States, 21% were resistant to penicillin, tetracycline, cefoxitin, or spectinomycin.[2] Although all of the isolates tested were sensitive to ceftriaxone, there have been reports of decreasing sensitivity to ceftriaxone among isolates from Southeast Asia.[3] Thus the current recommendation by the Centers for Disease Control to treat all gonococcal cervicitis and urethritis with ceftriaxone continues to be appropriate.[4] However, the current widespread use of ceftriaxone necessitates continued monitoring of isolates for resistance to ceftriaxone.

Chlamydia trachomatis is considered the most common sexually transmitted organism. Like *N. gonorrhoeae,* it is responsible for pelvic inflammatory disease, ectopic pregnancy, and infertility, in addition to neonatal conjunctivitis and pneumonitis. *C. trachomatis* and *N. gonorrhoeae* may be found as coinfections in 30% to 60% of patients, and up to 15% of *N. gonorrhoeae* may be

From the Section of Infectious Diseases, Department of Obstetrics and Gynecology, Baylor College of Medicine.
Reprint requests: Sebastian Faro, MD, PhD, Section of Infectious Diseases, Baylor College of Medicine, 6550 Fannin St., ST. 701, Houston, TX 77030.
6/0/29323

resistant to tetracycline.[5] Therefore the regimen recommended by the Centers for Disease Control, that is, to treat cervical infection with à combination of ceftriaxone plus doxycycline, seems appropriate.

The present study was undertaken to determine if the quinolone ofloxacin would be effective in treating cervical infection caused by *C. trachomatis*. Preliminary in vitro and in vivo data suggested that ofloxacin has activity against both *C. trachomatis* and *N. gonorrhoeae*,[6,7] whereas the quinolone ciprofloxacin does not appear to be especially effective in the treatment of chlamydial infection.[8] Ofloxacin is not affected by penicillin-binding proteins, penicillinase or β-lactamase, and so forth, and therefore would not be inhibited by penicillinase- or β-lactamase–producing or tetracycline-resistant strains of *N. gonorrhoeae*.

Methods

Patients attending the Harris County Gynecologic Clinic (low income) who were 18 years of age or older and positive for cervical infection with *C. trachomatis* alone or in conjunction with *N. gonorrhoeae* were randomized to receive treatment with either ofloxacin or doxycycline. Ofloxacin was administered in a dose of 300 mg, orally, twice a day, and doxycycline, 100 mg, was given orally, twice daily, for 7 days. Antibiotics were assigned according to a computer-generated randomization schedule provided by the Ortho Pharmaceutical Corp. Before enrollment into the study, all patients gave written informed consent.

On admission to the study, a complete medical history was obtained and a physical examination was performed. Venous blood was obtained for a complete blood count, white blood cell differential, platelet count, serum glucose, alkaline phosphatase, creatinine, blood urea nitrogen, total bilirubin, and aspartate aminotransferase. A urinalysis was also performed.

Specimens were obtained from the endocervix by gently wiping away vaginal discharge from the portio of the cervix with a large procto swab. A Dacron swab was then inserted into the endocervical canal, rotated in a clockwise manner for 30 to 40 seconds, and withdrawn. The initial swab was immediately placed into chlamydial transport medium, 2-SP (sucrose-phosphate) obtained from Bartels Immunodiagnostics, Bellevue, Wash. The specimens were immediately taken to the laboratory and processed for inoculation of cycloheximide-treated McCoy cells.[9] Inclusions were identified with monoclonal fluorescent antibody (obtained from Ortho Diagnostic Systems, Raritan, N.J.). A second swab was used to obtain a specimen for *N. gonorrhoeae* from the endocervix. This specimen was immediately streaked onto modified Thayer-Martin medium containing less than 3 μg of vancomycin. The inoculated plates were incubated at 35° C in 5% carbon dioxide environment. Isolates were confirmed by standard microbiologic methods. A Gram's stain was also performed on a specimen obtained from the endocervix.

The specimens were obtained from the endocervix with sterile cotton-tipped applicators and placed in *Mycoplasma* and *Ureaplasma* transport media (2-SP). The specimens were then inoculated on A-7 media (Scott Laboratories, Fiskeville, R.I.) and incubated at 35° C under anaerobic conditions.

Patients found positive for *C. trachomatis* and also those coinfected with *N. gonorrhoeae* were enrolled into the study. After completion of either antibiotic, patients were asked to return for follow-up evaluation between days 12 and 16 and days 28 and 35. At both visits pelvic examinations were performed, and endocervical specimens were obtained for test of cure. Repeat blood tests and urinalysis were obtained on the first follow-up visit only, unless an abnormal test was obtained.

Patients were considered cured if the follow-up culture for *C. trachomatis* and *N. gonorrhoeae* (if present originally) did not find the organism. A treatment failure was defined as persistence of the original offending pathogen on first follow-up visit. Patients who failed to complete the prescribed course of antibiotics, failed to return for follow-up evaluation, or took an additional antibiotic other than ofloxacin or doxycycline were considered nonevaluable. Only one patient was dropped from the study because she was 17 years old.

Sexual partners were afforded the opportunity of treatment with oral doxycycline, 100 mg, twice daily, for 7 days. Patients were given a supply a condoms and asked to use them for the duration of the study. In addition to the use of condoms, oral contraceptives were usually found to be used by most of the patients. All patients had a negative pregnancy test result before enrollment.

Patient compliance was assessed by having the patients return their pill container for pill counts.

Results

A total of 40 evaluable patients were enrolled into the study. Most patients were black (58%), 20% were Hispanic, and 22% were of other racial or ethnic backgrounds. The average age of the study population was 19 years. Although the population was relatively young (18 to 21 years of age), 55% of the patients had been pregnant at least once. Approximately 93% of the patients used some form of birth control, with 85% taking oral contraceptives. The study population reported a previous history of 19 episodes of sexually transmitted disease.

Among the 40 patients who had positive findings of *C. trachomatis*, only three also harbored *N. gonorrhoeae* (7.5%). All patients treated with ofloxacin were cured

Table I. Microbiologic results

	Chlamydia		*Neisseria*		*Mycoplasma*		*Ureaplasma*	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
Ofloxacin	20	0	1	0	10	5	14	13
Doxycycline	20	2	2	0	11	9	12	9

of the *C. trachomatis* infection (100% eradication), whereas 18 of 20 (90%) patients treated with doxycycline were cured (Table I). All three patients found to be infected with *N. gonorrhoeae,* one treated with ofloxacin and two with doxycycline, were cured.

Mycoplasma was found in 10 (50%) of the patients treated with ofloxacin. *Mycoplasma* was eradicated from half of the patients treated with ofloxacin. Approximately 55% (four patients) of those treated with doxycycline were *Mycoplasma* positive. Only two patients (10%) were found to be free of *Mycoplasma* after treatment with doxycycline. *Ureaplasma* was found in 70% of ofloxacin-treated patients and 60% of those treated with doxycycline. Neither antibiotic was very effective in eradicating *Ureaplasma* from the endocervix (Table I).

Comment

Although the study reported here does not contain a large number of patients, it demonstrates that ofloxacin is a suitable agent for the treatment of *C. trachomatis.* In fact, ofloxacin was as effective as doxycycline in eradicating *C. trachomatis* from the endocervix. These results were similar to those reported by other investigators in the treatment of female pelvic infections caused by *C. trachomatis.*[10, 11]

Mycoplasma and *Ureaplasma* are common inhabitants of the female lower genital tract. Their role as pathogens has not been well established, because these organisms have been retrieved frequently from healthy vaginas. However, they have been implicated in chorioamnionitis, bacterial vaginosis, infertility, and abdominal wound infections after cesarean section.[12-17] Ofloxacin was no better than doxycycline in eradicating *Mycoplasma hominis* or *Ureaplasma urealyticum* from the lower genital tract in the present study. Based on this data, neither of these agents would be suitable for eradicating either of these cell wall-less bacteria from the endocervix.

Although only three patients in the present study had a coinfection with *N. gonorrhoeae,* the one patient treated with ofloxacin was found to be free of both *C. trachomatis* and *N. gonorrhoeae* on posttherapy evaluation. In other studies containing large numbers of patients ofloxacin was found to be very effective in eradicating *N. gonorrhoeae.*[18-20] The accepted standard of treatment for uncomplicated cervical infection by *N. gonorrhoeae* or *C. trachomatis* is to administer a regimen of antibiotics effective against both of these organisms. The Centers for Disease Control has recommended the administration of 250 mg of ceftriaxone intramuscularly, followed by 100 mg of doxycycline, orally, twice daily for 7 days.[4] The use of ceftriaxone has been advised because of the increasing isolation of *N. gonorrhoeae* strains resistant to penicillin, tetracycline, cefoxitin, or spectinomycin. The national surveillance data revealed that resistant strains of *N. gonorrhoeae* are being isolated across the United States.[2] Because antimicrobial-resistant strains of *N. gonorrhoeae* are becoming widespread, with approximately 21% of strains being resistant to penicillin, tetracycline, cefoxitin, or spectinomycin, and because *C. trachomatis* is frequently found in conjunction with *N. gonorrhoeae,* ofloxacin appears to be a likely alternative and perhaps first-line antibiotic for the treatment of both of these common sexually transmitted organisms. The use of a single antibiotic should be more economic, administration is easier, and there is virtually no concern for resistance since resistance to quinolones is different than that to penicillins and cephalosporins.

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Susceptibility of female pelvic pathogens to oral antibiotic agents in patients who develop postpartum endometritis

Mark G. Martens, MD, Sebastian Faro, MD, PhD, Maurizio Maccato, MD, Gerald Riddle, MS, and Hunter A. Hammill, MD

Houston, Texas

Fifteen hundred patients were enrolled in a prospective, randomized study on the effect of antibiotic prophylaxis during cesarean section. Two hundred thirty-one patients developed postpartum endometritis, and the isolates obtained from the endometrium were tested for sensitivity to ampicillin, cefuroxime, ofloxacin, ciprofloxacin, and clindamycin. Minimum inhibitory concentrations of 50% and 90% of ampicillin, cefuroxime, and clindamycin were similar to previously reported values; however, slight differences were noted in the activity of the two quinolones to common pelvic isolates. The minimum inhibitory concentrations of 90% of ofloxacin and ciprofloxacin to 119 isolates of *Enterococcus faecalis* were 4.0 and 2.0, to 17 isolates of *Staphylococcus aureus* 1.0 and 0.5, to 39 isolates of *Escherichia coli* 0.5 and 1.0, to 46 isolates of *Bacteroides bivius* 4.0 and 8.0, to 57 isolates of *Gardnerella vaginalis* 1.0 and 2.0, to 71 isolates of *Staphylococcus epidermidis* 0.5 and 0.5, to 16 isolates of *Proteus mirabilis* 0.25 and 0.12, and to 50 isolates of *Lactobacillus* species 32.0 and 8.0 μg/ml, respectively. In summary, the quinolones have activity comparable with a variety of other oral agents versus female pelvic pathogens, with the quinolones ofloxacin and ciprofloxacin having better activity against most of the gram-negative isolates. Anaerobic activities were comparable with the β-lactams, but inferior to clindamycin and metronidazole as expected. (AM J OBSTET GYNECOL 1991;164:1383-6.)

Key words: Oral antibiotics, susceptibility, pelvic pathogens

From the Section of Infectious Diseases, Department of Obstetrics and Gynecology, Baylor College of Medicine.
Reprint requests: Mark G. Martens, MD, Associate Professor, Division of Infectious Diseases, Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX 77550.
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Associated with the rising number of cesarean sections being performed is a corresponding increase in the number of women developing postpartum endometritis.[1] Investigations that have studied the microbiology of endometritis have demonstrated that mul-

Table I. Endometrial isolates in 231 infected patients

Species	*No. of isolates*	*% of patients with species*
Gram-positive	257	
E. faecalis	119	52
Staphylococcus epidermidis	71	31
Lactobacilius sp.	50	22
Staphylococcus aureus	17	7
Gram-negative		
G. vaginalis	57	25
E. coli	39	17
Proteus mirabilis	16	7
Enterobacter cloacae	5	2
Enterobacter aerogenes	4	2
Klebsiella pneumoniae	5	2
Anaerobes	55	—
B. bivius	46	20
Fusobacterium nucleatum	5	2
B. fragilis	4	2

tiple bacterial species are often involved, with a mean number of two to four aerobic and anaerobic organisms recovered from each patient.[2]

In an effort to treat these polymicrobial infections, physicians have often relied on a combination of parenteral agents, such as clindamycin plus an aminoglycoside.[2] Single-agent alternatives, such as cefoxitin, ceftizoxime, mezlocillin, and piperacillin, have been used successfully.[3-5] Although most of these infections respond to parenteral agents alone, exact oral equivalents are often unavailable for patients who require extended therapy (such as patients with bacteremia, those with limited intravenous access, or those with abscesses).

This investigation examines the susceptibilities to several oral agents of organisms isolated from the endometrium of patients with endometritis after cesarean section. The newer oral antibiotics may provide a more economic alternative to parenterally administered agents where indicated.

Material and methods

Fifteen hundred patients were enrolled in a prospective randomized study on the effects of antibiotic prophylaxis with several β-lactam regimens during cesarean section. Eighty percent of the patients received a cephalosporin regimen, and 20% received a penicillin class agent as prophylaxis. At the time of diagnosis of postpartum endometritis, endometrial cultures were obtained by placement of a sterile speculum into the vagina, and the cervix was cleansed with a povidone iodine solution. The cervix was gently dilated, and an endometrial suction device (Pipelle, Unimar, Wilton, Conn.) was placed in the endometrial cavity to the uterine fundus. The tissue sample removed was placed into anaerobic transport agar (BBL, Cockeysville, Md.). All three specimens were cultured for aerobic and anaerobic organisms as well as *Mycoplasma, Ureaplasma,* and *Chlamydia* species with routine techniques as described previously.[6] Susceptibility testing was performed by microtube dilution methods (BBL Sceptor, Towson, Md.), and percent susceptibility was determined by minimum inhibitory concentration (MIC) interpretive standards from the National Committee for Clinical Laboratory Standards.[7]

Results

Two hundred thirty-one of the 1500 patients enrolled (15.4%) developed evidence of postpartum endometritis. Four hundred thirty-eight isolates were isolated for an average of 1.9 aerobic or anaerobic isolates per patient. *Enterococcus faecalis* was the most common gram-positive isolate and was isolated in 119 patients (52%). *Gardnerella vaginalis* was the most common gram-negative isolate and was found in 57 patients (25%). *Bacteroides bivius* was the most common anaerobic isolate and was present in 46 (20%) of patients diagnosed with endometritis. The remainder of the isolates are listed in Table I.

The MICs of 50% and 90%, MIC_{50} and MIC_{90}, respectively, are listed in Tables II through IV. Because the human pharmacokinetics of the tested antibiotics vary greatly, dosing regimens and achievable serum levels are also quite varied. To compare the activity of various antibiotics versus a specific pathogen, the MICs are used to create a percentage susceptibility table. Table V combines both the percentage susceptible and those designated as moderately susceptible.

Comment

Postpartum endometritis represents one of the most common pelvic infections. Soft tissue infections of the female genital tract are the result of upper genital tract inoculation from the cervicovaginal flora. Therefore lower genital tract organisms, such as *E. faecalis, G. vaginalis,* and *B. bivius,* become frequent pathogens in infections such as endometritis. The preponderance of the gram-positive isolates and non-fragilis strains of *Bacteroides* is consistent with most recent investigations of postoperative bacteriology in patients receiving β-lactam prophylaxis.[2,3,6] Because of the polymicrobial nature of pelvic infections, therapy is often initiated with a combination of antibiotics such as clindamycin or metronidazole plus an aminoglycoside or a broad-spectrum single agent. Although these infections are frequently eliminated with these intravenous agents without the need for follow-up oral antibiotics, oral agents may sometimes be indicated or necessary for patients with limited intravenous access or those tolerating oral nourishment and complicated infections requiring prolonged therapy such as bacteremia, ab-

Table II. MIC_{50} and MIC_{90} of gram-negative bacteria

	E. coli (*n* = 39)	*K. pneumoniae* (*n* = 5)	*P. mirabilis* (*n* = 16)	*G. vaginalis* (*n* = 57)	*Enterobacter sp.* (*n* = 5)	*E. aerogenes* (*n* = 4)
	MIC_{50}/MIC_{90}	MIC_{50}/MIC_{90}	MIC_{50}/MIC_{90}	MIC_{50}/MIC_{90}	MIC_{50}/MIC_{90}	MIC_{50}/MIC_{90}
Ofloxacin	0.5/1*	0.12/0.5	0.25/0.5	1/2	0.25/0.5	0.25/0.5
Ciprofloxacin	0.5/1	0.06/0.12	0.06/0.25	1/2	0.06/0.25	0.06/0.25
Ampicillin	4/32	16/16	2/16	0.5/2	32/34	16/32
Cefuroxime	2/4	4/8	1/8	1/2	4/8	4/8
Clindamycin	8/16	2/8	8/16	8/16	8/16	16/16

*μg/ml.

Table III. MIC_{50}/MIC_{90} of gram-positive bacteria

	S. faecalis (*n* = 119)	*S. aureus* (*n* = 17)	*S. epidermidis* (*n* = 71)	*Lactobacillus* (*n* = 50)
	MIC_{50}/MIC_{90}	MIC_{50}/MIC_{90}	MIC_{50}/MIC_{90}	MIC_{50}/MIC_{90}
Ofloxacin	2/4*	0.5/1	0.5/1	4/32
Ciprofloxacin	2/2	0.5/1	0.5/1	4/8
Ampicillin	1/1	1/4	0.25/8	0.5/1
Clindamycin	8/8	0.25/0.5	0.25/0.5	0.5/1
Cefuroxime	128/128	1/2	1/8	1/4

*μg/ml.

scess, phlegmon formation, septic pelvic thrombophlebitis, and pyelonephritis.

Oral equivalents for the aminoglycoside-containing regimens are not readily available. Agents comparable with the parenteral β-lactam agents are unavailable, with the possible exception of amoxicillin-clavulanic acid. Therefore the choice of an oral agent should be based on matching its spectrum of activity with the presumed or isolated pelvic pathogen or pathogens. From the direct culturing of the endometrium in patients diagnosed with endometritis after cesarean, it appears that the following organisms are most frequently isolated: *E. faecalis, G. vaginalis, B. bivius,* and *Escherichia coli.* In addition, coverage should also be provided for *Streptococcus agalactiae.*

According to the susceptibility testing of these isolates, coverage of these organisms with ampicillin is poor in many areas including the *Enterobacteriaceae,* the anaerobes, and *Staphylococcus* species. Therefore its use should be limited to enterococcal infections and isolates tested as susceptible.

Clindamycin and metronidazole had excellent activity against anaerobes as expected; however, metronidazole has little aerobic coverage, and thus it was not tested against aerobic organisms. Clindamycin demonstrated good activity against gram-positive organisms except for the enterococci, but has little gram-negative activity.

Cefuroxime axetil, a new oral second-generation cephalosporin, is the oral equivalent to intravenous cefuroxime. Its good gram-negative activity may make it an appropriate oral agent in gram-negative infections

Table IV. MIC_{50}/MIC_{90} of anaerobes

	B. fragilis (*n* = 4)	*B. bivius* (*n* = 46)	*F. nucleatum* (*n* = 5)
	MIC_{50}/MIC_{90}	MIC_{50}/MIC_{90}	MIC_{50}/MIC_{90}
Ofloxacin	2/4*	4/8	1/2
Ciprofloxacin	2/4	8/8	2/4
Ampicillin	16/32	32/32	1/2
Clindamycin	1/1	0.5/1	0.5/0.5
Cefuroxime	32/128	16/32	2/16
Metronidazole	2/4	2/2	—

*μg/ml.

such as pyelonephritis or soft tissue infections with susceptible organisms, especially in pregnant patients in whom the quinolones are contraindicated.

Of all the agents tested, the quinolones—ofloxacin and ciprofloxacin—demonstrated the broadest range of activity. Their gram-negative activity was superior to all other agents, with the activity of ofloxacin and ciprofloxacin essentially equivalent. The gram-positive activity was also excellent; however, there was slightly better activity by ampicillin versus the enterococci. Although activity was much poorer for the quinolones against the lactobacilli, this still may be beneficial; Faro et al.[8] have described the protective benefits of the lactobacilli in maintaining a balanced flora.

The quinolones had only moderate activity versus the anaerobes; ofloxacin maintained a distinct advantage over ciprofloxacin, with 75% and 39% susceptibility for *B. fragilis* and *B. bivius* for ofloxacin, and only 50% and 2% susceptibility with ciprofloxacin. However, only

Table V. Percent susceptible

Bacteria (No. tested)	*Ofloxacin*	*Ciprofloxacin*	*Ampicillin*	*Cefuroxime*	*Clindamycin*	*Metronidazole*
E. coli (39)	100	99	56	100	0	—
K. pneumoniae (5)	100	100	100	80	60	—
P. mirabilis (16)	100	100	88	94	0	—
G. vaginalis (57)	95	93	100	100	97	—
E. cloacae (5)	100	100	0	80	0	—
E. aerogenes (4)	100	100	50	100	0	—
B. fragilis (4)	75	50	50	25	100	100
B. bivius (46)	39	2	33	59	100	100
F. nucleatum (5)	80	80	100	80	100	—
E. faecalis (119)	75	93	100	6	2	—
S. aureus (17)	100	100	24	100	100	—
S. epidermidis (71)	100	99	50	96	93	—
Lactobacillus sp. (50)	36	44	100	100	100	—

B. bivius were found with enough frequency to make strong comparisons. In documented anaerobic infections, though, the bulk of the previous literature still suggests the use of clindamycin or metronidazole.

In summary, the quinolone class of antibiotics, ofloxacin and ciprofloxacin, demonstrated a broader range of activity versus female genital tract isolates over the previously studied oral antibiotics ampicillin, cefuroxime, clindamycin, and metronidazole. Although investigations need to be performed to ascertain if use of the oral quinolones can replace empiric intravenous therapy of female genital tract infections, their use in areas in which oral therapy is currently used is supported by their in vitro activity versus common organisms such as *E. faecalis, B. bivius, G. vaginalis,* and *E. coli.*

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New directions in the diagnosis and therapy of urinary tract infections

Robert D. Powers, MD
Charlottesville, Virginia

Urinary tract infections are among the most common bacterial infections. To provide appropriate and cost-effective treatment, physicians need to stratify patients with urinary complaints into uncomplicated or complicated categories. This can be accomplished by the history, presenting symptoms, risk factors, and physical examination. Complicated urinary tract infections occur in patients with a history of recurrent infections, signs or symptoms of upper tract disease, or coexisting conditions such as pregnancy, immunosuppression, or structural anomalies of the urinary tract. Uncomplicated urinary tract infections occur in otherwise healthy women who have a history of lower tract symptoms of short duration. Symptoms of urinary tract infection include some combination of dysuria, frequency, urgency, hematuria, and suprapubic pain. An uncomplicated urinary tract infection is not accompanied by fever or flank pain. The microbiology of uncomplicated urinary tract infection is predictable, with *Escherichia coli* and other *Enterobacteriaceae, Staphylococcus saprophyticus,* and *Enterococcus* causing more than 90% of urinary tract infections. A history, brief physical examination, and urinalysis are all that is necessary to diagnose a urinary tract infection. Some of the specialized dipsticks and rapid screens are as accurate as microscopic examination in detecting urine white cells. A presumptive diagnosis can be made when a patient has clinical symptoms and some combination of pyuria, hematuria, or bacteriuria. Urine cultures are unnecessary in uncomplicated urinary tract infections and add substantially to the cost of therapy. Pitfalls in the diagnosis include other entities causing dysuria, such as vaginitis, vulvar lesions, physical or chemical irritants, and sexually transmitted diseases. Appropriate therapy requires selection of a drug and determination of the length of treatment. A minor infection should be treated with easy, safe, cost-effective therapy. For urinary tract infections, there are too many antibiotic options, ranging from a single, parenteral dose to a 14-day course of oral medication. Early optimism about single-dose oral therapy has been replaced by evidence suggesting that 3 days of therapy is probably the best. This will eradicate simple urinary tract infections in virtually all patients and decrease the incidence of relapse, whereas patients who are treatment failures usually have occult upper tract infection. Drug choices for short-course therapy include representatives from the penicillin, sulfa, and quinolone families. Selection of a specific drug requires consideration of costs, allergies, side effects, and spectrum of activity. A knowledge of local microbial sensitivity profiles and individual patient tolerance is helpful in guiding the clinician to the appropriate therapeutic regimen. (Am J Obstet Gynecol 1991;164:1387-9.)

Key words: Urinary tract infection, urethritis, vaginitis, dysuria

Urinary tract infections (UTIs) are among the most common bacterial illnesses seen by physicians. They affect 10% to 20% of women in the United States annually and prompt 5.2 million office visits per year. Total annual U.S. health care expenditures for outpatients with UTIs approach 1 billion dollars; additional millions are spent on the 100,000 patients hospitalized with UTIs yearly.

It is important for the physician in clinical practice to have a rapid, cost-effective, yet comprehensive approach to female patients with urinary tract complaints. Accurate diagnosis leads to appropriate therapy, which typically involves the administration of oral antimicrobial agents. Numerous effective drugs are available for the treatment of UTIs, and thus the clinician must have an effective way of narrowing the options for each patient. During the past few years, research into appropriate diagnostic and therapeutic regimens has simplified the approach to UTIs by doing away with some time-honored practices.

From the University of Virginia School of Medicine.
Reprint requests: Robert D. Powers, MD, Director, Emergency Medical Services, University of Virginia Health Sciences Center, Box 523-21, Charlottesville, VA 22908.
6/0/29044

Approach to the patient with a UTI

Women with UTIs typically have dysuria. Because dysuria has a number of origins (Table I), the initial history and physical examination should be directed at defining the source of the complaint. In addition to UTI, other disorders of the urethra, vagina, and perineum must be considered in the evaluation of women with dysuria. Usually the urinary tract can be isolated as the source of dysuria by taking a careful history. If

Table I. Causes of dysuria

UTI: Lower or upper tract
Urethritis: *Chlamydia*, gonorrhea, herpesvirus
Vaginitis: Bacterial vaginosis, *Trichomonas*, yeast
Perineal lesions: herpesvirus, other sexually transmitted diseases, dermatologic diseases
Physical or chemical irritants: Diet, occupational exposures

there are inconsistent or confusing historical features, examination of the perineum and pelvic organs is essential for accurate diagnosis.

Once it has been established that the dysuria originates in the urinary tract, the next step is to make the distinction between complicated and uncomplicated infection. Uncomplicated UTI (or acute cystitis) is a bacterial infection of the bladder occurring in an otherwise healthy woman. These patients generally have a short duration of symptoms, including some combination of dysuria, frequency, urgency, hematuria, and suprapubic pain or tenderness. Signs and symptoms of UTI (fever, flank pain, or tenderness) are absent. Patients with signs and symptoms of UTI, or those who are immunocompromised are considered to have complicated UTIs. Men, pregnant women, and those with recurrent infections or structural or functional anomalies of the urinary tract also fall into the complicated UTI category.

Patients with complicated UTIs require urine culture and sensitivity determinations and a longer, more aggressive course of treatment. Although hospitalization is not always necessary, close follow-up is essential in these patients to prevent morbidity associated with invasive infection. In addition to a 7- to 10-day course of an appropriate oral antimicrobial agent, "sequential" therapy may be indicated. This involves initial treatment with a parenteral dose of gentamicin or ceftriaxone before the initiation of outpatient therapy. Conversely, uncomplicated UTIs can be treated effectively with a short course of oral antimicrobial agents, and usually do not require much in the way of laboratory evaluation.

Laboratory test use in UTI

In a patient who appears by history and physical examination criteria to have an uncomplicated UTI, a urinalysis is all that is necessary for laboratory confirmation of the diagnosis. Examination of spun urine should disclose numerous white blood cells and bacteria. The newer dipsticks that test for leukocyte esterase and nitrite may substitute for a urinalysis if interpreted correctly. When the results of a dipstick test are positive for both leukocyte esterase and nitrite in the appropriate clinical setting, a microscopic examination of the urine is probably unnecessary. Blood tests such as a complete blood count are costly and are not helpful in the diagnosis or therapy of uncomplicated UTI.

Table II. When urine culture is appropriate

Complicated UTIs
Uncertain clinical or urinalysis features
Relapse
Recent hospitalization or invasive procedure

Whether to culture a urine specimen in a patient with a UTI is an important consideration given the cost/benefit ratio. The cost to the patient for a urine culture with sensitivities is about $40, and in many cases results will become available long after the patient is cured. Accordingly, urine culture is not recommended for most patients with uncomplicated UTI but should be reserved for those patients (Table II) in whom the situation is complicated or confusing. Despite the classic teaching that 10^5 colony forming units per milliliter are necessary for a positive urine culture, recent evidence indicates that a true positive culture can have as low as 100 colony forming units per milliliter of bacteria.

Treatment of UTI

Once the diagnosis of UTI has been established and the patient is assigned to either the complicated or uncomplicated category, the choice of an antimicrobial agent must be made. Because most UTIs will be treated empirically, a knowledge of the likely pathogens is necessary to guide selection of appropriate therapy. Most UTIs are caused by species of gram-negative rods (Table III), with about 10% caused by *Enterococcus* or *Staphylococcus saprophyticus*. There may be regional or local variations to this list, and it is important for clinicians to know their community profile of causative organisms and their sensitivities. This information is available from hospital or commercial microbiology laboratories. The goal of therapy for UTI is to eradicate the infecting organism while causing minimal systemic or local side effects. Allergic reactions are a concern, as are the development of yeast vaginitis or selection of resistant flora in the gastrointestinal tract. For a regimen to be effective in large numbers of patients, it should be low cost and have a convenient dosage schedule.

Numerous antimicrobial agents are appropriate for oral therapy of UTIs (Table IV). Most of the common pathogens are sensitive to sulfa derivatives and cephalosporins. Ampicillin and amoxicillin are no longer considered optimal choices because of a 20% to 30% resistance in *Escherichia coli*.

The principal new development in the treatment of UTIs is the release of the quinolone class of antibiotics. These nalidixic acid derivatives have excellent activity against a broad range of urinary tract pathogens, and the drugs are well tolerated by patients. Norfloxacin, ofloxacin, and ciprofloxacin are presently available; norfloxacin is approved only for UTIs since it is heavily concentrated in the urine. Ciprofloxacin and ofloxacin are effective against a wide variety of bacterial infec-

Table III. Microbiology of UTIs

80% *E. coli*
10% *Klebsiella* sp, *Proteus* sp, *Enterobacter* sp, *Pseudomonas* sp
10% Gram-positive: *S. saprophyticus, Enterococcus*
Note: Culture positive 10^2-10^5 CFU/ml

tions, including those involving the urinary tract. Numerous other quinolone compounds are in various stages of development. The principal advantage they offer over more conventional agents for UTIs is increased activity against *Pseudomonas* species.

At present trimethoprim/sulfamethoxazole is probably the best combination of efficacy and cost for treatment of uncomplicated UTIs. The quinolones, cephalexin, and nitrofurantoin offer excellent alternatives when allergy, patient preferences, or past failures are important. These agents have excellent clinical efficacy but are two to three times more expensive than trimethoprim/sulfamethoxazole.

Duration of antibiotic therapy is an important consideration in UTI. The classic teaching that a 7- to 10-day course is necessary has largely been abandoned, and these long courses should be reserved for patients with complicated UTI or failure of a shorter regimen. Initial optimism about the use of single-dose therapy has been tempered by an unacceptable failure rate in certain populations. As such, a short course of 3 to 5 days is advisable for the great majority of women with uncomplicated UTIs. Trimethoprim/sulfamethoxazole, ciprofloxacin, and norfloxacin can be given twice-daily for short course treatment. Ofloxacin may offer once-daily therapy for short-course treatment of uncomplicated UTIs because of its extended half-life and increased serum and urine levels.

Follow-up of patients with uncomplicated UTIs should be symptom driven. In a private office practice, the cure rate for short-course therapy of uncomplicated UTI should approach 90%. Some patients who failed to respond to therapy have occult upper tract UTIs and must be treated with a longer course tailored to the infecting organism. Noncompliant patients or those with a resistant organism will have recurrent or persistent symptoms and merit further diagnostic analysis.

Summary

Women with UTIs can be stratified into uncomplicated or complicated groups by simple historical and physical examination criteria. Those patients with complicated UTIs require a combination of parenteral and oral therapy or a prolonged (7 to 14 days) course of oral antimicrobial agents. Uncomplicated cystitis can be treated with 3 to 5 days of inexpensive, well-tolerated antibiotics, giving a very high cure rate. Of the agents currently available, trimethoprim/sulfamethoxazole probably offers the best combination of efficacy and cost-effectiveness. Quinolones provide an expanded field of alternatives for the safe and effective treatment of all types of UTI. Looking ahead into the 1990s, the recent release of ofloxacin and the expected approval of other compounds will increase the number of quinolone agents available for clinical use. Ultimately, a variety of quinolones will be available, giving clinicians a broader range of effective compounds from which to choose. Individual differences in antimicrobial spectrum and pharmacokinetics will distinguish the quinolones from one another, and the variety will better enable physicians to tailor therapy to a specific clinical situation.

Table IV. Oral antimicrobial choices for UTIs

Penicillin derivatives
Ampicillin
Amoxicillin
Cephalosporins
Quinolones
Ciprofloxacin*
Norfloxacin*
Ofloxacin*
Fleroxacin
Enoxacin
More on the way
Sulfa drugs
Sulfisoxazole
Sulfamethoxazole
Miscellaneous/combinations
Nitrofurantoin
Trimethoprim
Trimethoprim/sulfamethoxazole
Amoxicillin/clavulanic acid

*Commercially available.

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A randomized trial of ofloxacin versus cefoxitin and doxycycline in the outpatient treatment of acute salpingitis

George D. Wendel, Jr., MD, Susan M. Cox, MD, Roger E. Bawdon, PhD, Sheri K. Theriot, RNC, Molly C. Heard, MA, and Brenda J. Nobles

Dallas, Texas

The object of this randomized study was to compare the safety and efficacy of oral ofloxacin, 400 mg twice daily for 10 days, versus intramuscular cefoxitin, 2 gm, plus oral probenecid, 1 gm, followed by oral doxycycline, 100 mg twice daily for 10 days, in the outpatient treatment of uncomplicated acute salpingitis. Thirty-eight women (53%) had *Neisseria gonorrhoeae* from their pretreatment endocervical or endometrial cultures, and 18 had *Chlamydia trachomatis* (25%). Thirty-five of 37 women (95%) treated with the ofloxacin regimen were clinically cured, and 34 of 35 (97%) were cured with the cefoxitin-doxycycline regimen ($p = 0.52$). One clinical failure occurred in each group with *N. gonorrhoeae* infection, and one failure occurred in the ofloxacin group because of side effects. The bacteriologic response for *N. gonorrhoeae* in both groups was 100%. The eradication of *C. trachomatis* was 100% (10/10) for the cefoxitin/doxycycline group and 86% (6/7) for ofloxacin. The side effects were similar in both groups of subjects. In this study both regimens were effective for the outpatient treatment of uncomplicated acute salpingitis. (Am J Obstet Gynecol 1991;164:1390-6.)

Key words: Ofloxacin, acute salpingitis, *N. gonorrhoeae*, *C. trachomatis*

There is no single, oral agent with adequate efficacy in the treatment of acute salpingitis,[1, 2] a polymicrobial infection most commonly caused by *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and other aerobic and anaerobic pathogens.[3-5] Although many patients are hospitalized for treatment, most women are treated for acute salpingitis as outpatients.[6] Patients with mild or uncomplicated salpingitis (without diffuse peritonitis or pelvic abscess)[7] who are able to tolerate oral medications are generally considered candidates for ambulatory therapy. Prior investigations have revealed the limitations of penicillin plus ampicillin, doxycycline, and tetracycline in the outpatient therapy of acute salpingitis.[2, 8, 9] Current Centers for Disease Control–recommended therapeutic regimens include an agent with broad-spectrum efficacy against *N. gonorrhoeae*, other aerobes, and anaerobes plus an antichlamydial agent.[10] With inpatient therapy these can be administered simultaneously or sequentially. For the outpatient therapy of uncomplicated acute salpingitis, this combination is usually given sequentially: an intramuscular injection of an antigonococcal agent followed by the oral administration of an antichlamydial agent for approximately 10 days.[10]

Typically tetracycline or doxycycline is the oral agent of choice for outpatient treatment of *C. trachomatis* infection.[11] Recently antimicrobials such as amoxicillin and clindamycin have generated renewed interest in their antichlamydial activities.[12, 13] However, none of these agents have satisfactory clinical experience or efficacy to warrant their recommendation as a single agent for therapy of *C. trachomatis*. The newer fluoroquinolones have excellent in vitro sensitivities for a broad spectrum of pathogens, including *N. gonorrhoeae* and *C. trachomatis*.[14, 15] They can be administered orally and may be effective single agents for the treatment of sexually transmitted diseases.[16] Clinical trials have shown clinical and microbiologic responses in 98% to 100% of patients with gonorrhea[17-19] and 83% to 100% of patients with chlamydial infection treated with ofloxacin.[19-21] The purpose of this study was to compare the safety and efficacy of ofloxacin, a new fluorinated 4-quinolone, versus cefoxitin plus probenecid, followed by doxycycline, in the outpatient treatment of uncomplicated acute salpingitis.

Material and methods

This study was a prospective, randomized, open-label phase III clinical trial to evaluate the safety and efficacy of ofloxacin in the treatment of uncomplicated acute salpingitis. The study was approved by the University

From the Department of Obstetrics and Gynecology, University of Texas Southwestern Medical School and Parkland Memorial Hospital.

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Reprint requests: George D. Wendel, Jr., MD, Department of Obstetrics andGynecology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9032.

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Table I. Diagnostic inclusion criteria for uncomplicated acute salpingitis

All three of the following:
Direct lower abdominal tenderness with or without rebound tenderness
Cervical motion tenderness
Adnexal tenderness
Plus at least one of the following:
Recent positive endocervical culture for *N. gonorrhoeae* or *C. trachomatis*
Temperature >100.4° F
White blood cell count >10,000/mm³

Table II. Clinical severity score for grading acute salpingitis

The following signs were evaluated:
Lower abdominal direct tenderness
Adnexal tenderness
Pain on motion of cervix or uterus
According to the following grades:

Grade	Score	Discomfort
Severe	3	With minimal pressure
Moderate	2	Easily elicited by observation of facial expression
Minimal	1	Not evident by observation of facial expression
Absent	0	Inability to elicit any abnormal discomfort

Total score is equal to sum of value of each sign.
Maximum score = 9.

of Texas Southwestern Medical Center Institutional Review Board. The subjects were enrolled from the Obstetrics and Gynecology Emergency Room of Parkland Memorial Hospital after they gave informed consent between May 1, 1987, and Dec. 1, 1987.

Nonpregnant, nonlactating women using a reliable form of contraception who met the inclusion criteria for uncomplicated acute salpingitis[7] (Table I) were offered entry into the study. The patients were excluded from the study if their pelvic infection was severe enough to require parenteral antimicrobial therapy (diffuse peritonitis) or if surgical intervention within the next 24 hours was anticipated. In addition, patients were excluded if there was evidence of a pelvic abscess on sonographic or clinical examination.

All the patients were questioned about the duration of symptoms, past history of pelvic inflammatory disease, and possible allergy to study medications. In addition, all patients were given a clinical severity score at the time of admission according to a modification of previously described grading systems for acute salpingitis (Table II).[22, 23] All patients underwent a complete physical examination, and cultures were collected for *N. gonorrhoeae* and *C. trachomatis*. Endocervical cultures for *N. gonorrhoeae* plus *C. trachomatis* and gonococcal rectal cultures were collected on admission to the study. During the first half of the study period, all patients also had endometrial cultures for *N. gonorrhoeae* and *C. trachomatis*. Laboratory evaluation included a complete blood count, platelet count, white blood cell differential count, and erythrocyte sedimentation rate. Serum chemistry tests of renal function and liver function were also obtained as was a prothrombin time and a partial thromboplastin time. A urinalysis and pregnancy test were performed. All patients underwent pelvic sonography to rule out pelvic masses or abscesses before entry into the study.

After informed consent was obtained, subjects were assigned a randomized code number designating therapy, which was then administered in an open fashion. The two treatment regimens were instituted immediately in the emergency room area. An equal number of women was assigned to each treatment regimen. Patients received either ofloxacin, 400 mg orally every 12 hours for 10 days, or cefoxitin, 2 gm intramuscularly, and probenecid, 1 gm orally, followed by doxycycline, 100 mg orally every 12 hours for 10 days. Thus all patients received a 10-day course of outpatient therapy for acute salpingitis.

The subjects were instructed about the nature of their pelvic infection and the need for compliance to the therapeutic regimens. Their sexual partners were referred to the Dallas County Sexually Transmitted Disease Clinic for evaluation and treatment. The patients were instructed to abstain from coitus until completion of the study but were also given nonmedicated, nonlubricated condoms as a precaution. Patients were instructed to report any unprotected coitus at follow-up visits.

The subjects were asked to return for follow-up evaluation 3 to 4 days after entry into the study, at 13 to 15 days after entry into the study, and at 25 to 30 days after entry into the study. At the initial follow-up visit, patients were questioned about compliance and side effects but were not examined unless there were complaints. At the 13- to 15-day visit, endocervical and endometrial cultures for *N. gonorrhoeae* plus *C. trachomatis*, rectal *N. gonorrhoeae* cultures, the clinical severity score, and laboratory tests were repeated. The clinical response to therapy and the microbiologic response for *N. gonorrhoeae* were determined at this visit. At the 25- to 30-day follow-up visit, endocervical, endometrial, and rectal cultures and the clinical severity score were repeated; also, the microbiologic response for subjects with *C. trachomatis* was determined. An effort was made to ensure compliance with all three follow-up visits by telephone contact and registered mail for delinquent patients.

The clinical response compared with the examination at the time of admission of all evaluable patients was determined at the 13- to 15-day follow-up visit. A cure

Table III. Demographic comparison of study groups at admission

	Cefoxitin/doxycycline (N = 35)	*Ofloxacin (N = 37)*
Age (yr)	24.7	23.3
Parity	1.8	1.4
History of prior sexually transmitted disease (%)	37.1	48.6
Days of symptoms	2.9	3.7
Temperature (° F)	100.5	100.6
Clinical severity score	6.2	6.7
Height (inches)	63.2	62.8
Weight (lb)	134.6	128.3
WBC (per mm^3)	14,900	13,550
ESR (mm/hr)	37.6	42.1

WBC, White blood cell count; *ESR*, erythrocyte sedimentation rate.

Table IV. Microbiologic comparison of study groups at admission

Pathogen	*Cefoxitin-doxycycline (N = 35)*	*Ofloxacin (N = 37)*	*Total*
N. gonorrhoeae	10	17	27
C. trachomatis	4	3	7
N. gonorrhoeae and *C. trachomatis*	6	5	11
Negative culture	15	12	27

Table V. *N. gonorrhoeae* microbiologic susceptibility (μg/ml)

Drug	*Range*	MIC_{50}	MIC_{90}	*Resistant*
Cefoxitin	≤0.12-4	≤0.5	1	0
Doxycycline	≤0.12-8	0.5	2	1
Ofloxacin	≤0.03-1	≤0.12	0.25	0

MIC, Minimal inhibitory concentration.

was defined as complete resolution of tenderness (>65% decrease in clinical score). Clinical improvement was defined as partial resolution of tenderness (31% to 64% decrease in clinical score), and failure was defined as minimal or no improvement in tenderness (<30% decrease in clinical score). A microbiologic cure required eradication of all pathogens present at the time of admission. A microbiologic partial cure was eradication of only one pathogen with persistence of the other. A microbiologic failure was defined as a persistence or relapse of the original pathogen(s).

Cultures were obtained from the endocervical canal for *N. gonorrhoeae* and *C. trachomatis* by means of sterile Dacron applicators after endocervical mucus and pus were removed with a dry sterile swab. Endometrial cultures were obtained by a double-lumen catheter-protected brush (MediTech, Watertown, Mass.). The *N. gonorrhoeae* cultures were immediately plated on modified Thayer-Martin medium and incubated at 35° C in a 5% carbon dioxide environment. Isolates were confirmed by standard microbiologic methods. All the *N. gonorrhoeae* isolates were tested for the presence of β-lactamase by nitrocefin-impregnated disks (Cefinase, BBL Microbiology Systems, Cockeysville, Md.). *C. trachomatis* specimens were obtained after *N. gonorrhoeae* cultures. These specimens were then placed in a sterile transport media (Cultureset, Ortho Diagnostic Systems, Raritan, N.J.). The specimens were then transported to the laboratory within 15 minutes of collection for inoculation into cycloheximide-treated McCoy cells in tissue culture vials or stored at −70° C until cultures could be performed. The tissue cultures were performed according to a previously described protocol.[24]

Susceptibility testing of *N. gonorrhoeae* isolates to the three antimicrobials was performed with agar dilution methods in accordance with approved standards.[25] Bacterial resistance to cefoxitin, doxycycline, and ofloxacin was defined as a minimal inhibitory concentration of ≥16 μ/ml, ≥8 μg/ml, and ≥4 μg/ml, respectively. Susceptibility testing was performed for *C. trachomatis* only if there was a treatment failure.

A retrospective medical record review was performed in all subjects 3 years after the completion of the study to ascertain subsequent pregnancies. The review only included visits or admissions to Parkland Memorial Hospital. Voluntary infertility and sequelae, such as chronic pelvic pain and involuntary infertility, were not measurable.

Categoric data were analyzed for differences in frequency between the two treatment groups by means of χ^2 test. Continuous data were analyzed with a two-tailed Student t test. A p value of <0.05 was considered significant in the data analyses.

Results

Ninety-six women were enrolled in the study for treatment with the two study regimens. Twenty-four women were considered unevaluable because of total noncompliance with the study drug regimen (one woman) or lack of attendance at any follow-up visits (23 women). The evaluable study group consisted of 72 women: 35 who received the cofoxitin-probenecid and doxycycline regimen and 37 who received ofloxacin.

A comparison of the two study groups regarding age, race, marital status, parity, and a prior history of sexually transmitted diseases revealed no significant differences (Table III). A comparison of clinical and laboratory variables at the time of enrollment revealed no significant difference in days of symptoms before admission, temperature, clinical severity score, height, weight, white blood cell count, or erythrocyte sedimentation rate (Table III).

Results of a culture from the endocervix and endo-

Table VI. Clinical and microbiologic response

	Cefoxitin-doxycycline				*Ofloxacin*			
	Clinical response		*Microbiologic response*		*Clinical response*		*Microbiologic response**	
	Cured	*Failure*	*Cured*	*Failure*	*Cured*	*Failure*	*Cured*	*Failure*
N. gonorrhoeae	9	1	16	0	16	1	21	0
C. trachomatis	4	0	10	0	3	0	6	1†
N. gonorrhoeae plus *C. trachomatis*	6	0			4	1‡		
No pathogen	15	0			12	0		
TOTAL	34	1			35	2		

*Excludes treatment failure because of side effects.

†Patient with unprotected coitus and untreated sexual partner.

‡Considered treatment failure because of intolerable side effects.

metrium (or both) were positive in 63% (45/72) of patients (Table IV). The most common isolate was *N. gonorrhoeae*, which was found in 53% (38/72) of the women. Twenty-five percent (18/72) of the women had *C. trachomatis* from their genital cultures. Ten percent (7/72) of the women had *C. trachomatis* alone, and 15% (11/72) had both *N. gonorrhoeae* and *C. trachomatis* in their cultures. Overall 29% (11/38) of patients with *N. gonorrhoeae* had concomitant *C. trachomatis* infection. No significant differences were noted between the rates of isolation of *N. gonorrhoeae*, *C. trachomatis*, *N. gonorrhoeae* and *C. trachomatis*, or negative cultures when the two study groups were compared.

Forty-three (60%) of evaluable patients had endometrial cultures performed. There was concordance between the endocervical and endometrial cultures in 91% of the subjects (39/43). Three women had positive cervical cultures but no endometrial growth; one woman had only endometrial *C. trachomatis*. Overall 58% (25/43) of the subjects had positive endometrial cultures. Twenty-one (49%) women had gonococcal infection, and five (12%) had chlamydial infection of the upper tract. No significant difference was observed in the rates of upper tract infection between the two study groups.

All but one of the *N. gonorrhoeae* isolates were susceptible to the three study drugs. The strain was resistant only to doxycycline. The minimal inhibitory concentration ranges are shown in Table V. None of the isolates were β-lactamase–producing strains. Susceptibility testing was not performed for any *C. trachomatis* isolates.

Overall 96% (69/72) of patients were clinically cured after they completed the outpatient study regimens. Ninety-five percent (35/37) of the women treated with the ofloxacin regimen were cured, and 97% (34/35) of patients receiving the cefoxitin-probenecid and doxycycline regimen were cured (Table VI). In each group a woman with an endocervical and endometrial infection caused by *N. gonorrhoeae* was considered a clinical failure. One woman in the ofloxacin group with mixed gonococcal and chlamydial endocervical infection was counted as a failure because of side effects. She showed clinical improvement (follow-up severity score of 1 and negative cultures for *N. gonorrhoeae* and *C. trachomatis*) but severe nausea and vomiting after 3 days of treatment. The average clinical severity score at the 13- to 15-day visit in each group was 0.3, and the white blood cell counts were within the normal range for both groups.

Table VII. Clinical response in women with endocervical and endometrial cultures

Site of positive culture	*Cefoxitin-doxycycline*	*Ofloxacin*	*Total*
Endocervix	9/9	11/11	20/20
Endometrium	10/11	11/12	21/23
TOTAL	19/20	22/23	41/43

The response in women with gonococcal infection was 94% (15/16) for cefoxitin-doxycycline and 91% (20/22) for ofloxacin. The response in women with chlamydial infection was 100% (10/10) for cefoxitin-doxycycline and 88% (7/8) for ofloxacin. No significant difference in the clinical cure rate was seen in the group with positive endometrial cultures versus those with only positive endocervical cultures for the individual study groups (Table VII). No patient with only chlamydial infection or culture-negative infection was considered a failure.

The microbiologic response for *N. gonorrhoeae* and *C. trachomatis* is shown in Table VI. No woman had persistence of *N. gonorrhoeae* or *C. trachomatis* at the 13- to 15-day follow-up visit. Despite clinical failure in two women with gonococcal endocervical and endometrial infection and one with mixed endocervical infection, microbiologic eradication of the pathogens present at the time of admission occurred in each case.

Table VIII. Side effects in study groups

	Cefoxitin-doxycycline (N = 35)	*Ofloxacin (N = 37)*
Nausea	3	2
Thrombocytosis	2	0
Candida vaginitis	2	1
Eosinophilia	2	1
Monocytosis	1	0
Headaches	0	1
Allergy	0	1
TOTAL	9 (26%)	6* (16%)

*p = NS.

At the final follow-up visit at 25 to 30 days after entry into the study, one woman in the ofloxacin group had recurrent *C. trachomatis* and *N. gonorrhoeae* (Table VII). The subject had been clinically and microbiologically cured at her prior visit, and she admitted to unprotected coitus with untreated partners since the prior follow-up visit. She had a normal pelvic examination, free of signs of clinical pelvic infection or cervicitis. The minimal inhibitory concentrations for the recurrent *N. gonorrhoeae* isolates were identical to the strain from admission to the study. All the study subjects maintained normal clinical examinations at the final follow-up visit.

Nine percent (6/64) of the women had rectal *N. gonorrhoeae* infection, and eight women did not have rectal cultures taken. The three women in each treatment group resulted in a concomitant rectal infection rate of 16% (6/38) in those with *N. gonorrhoeae*. There were no treatment failures in either group at the 13- to 15-day follow-up.

Twenty-one percent (15/72) of patients developed side effects during treatment. Twenty-six percent (9/35) of patients treated with cefoxitin-probenecid followed by doxycycline developed side effects, and 16% (6/37) treated with the ofloxacin regimen developed side effects. There were no significant differences in the frequency of side effects in the two groups or in the type of side effects noted (Table VIII). One patient in the ofloxacin group developed severe nausea and vomiting early in therapy and was removed from the protocol. She was switched to a parenteral regimen as an inpatient and was considered a treatment failure because of side effects in the previous efficacy analysis.

Retrospective medical record review revealed that 87% (63/72) of the women had subsequent visits or admissions to Parkland Memorial Hospital. Eight women in the cefoxitin-doxycycline group and nine women in the ofloxacin group had prior tubal ligations; they were omitted from analysis. Intrauterine pregnancies occurred in 33% (9/27) of the subjects in the cefoxitin-doxycycline group and in 54% (15/28) of the ofloxacin group (p = 0.09). One additional ectopic pregnancy occurred in the ofloxacin group. Crude pregnancy rates were not adjusted for prior salpingitis, subsequent recurrent salpingitis, or voluntary infertility.

Comment

The antimicrobial regimens compared were equally effective in the outpatient treatment of women with uncomplicated pelvic inflammatory disease. Excellent clinical and bacteriologic efficacy were noted with both regimens in this group of patients. The clinical efficacy of both regimens was similar for patients with *N. gonorrhoeae* and *C. trachomatis* infection (or both), as well as for patients who had neither pathogen isolated. Because of the number of subjects evaluated and subsequent low statistical power, significant differences between the two regimens may have been obscured. The efficacy of therapy may also be related to the preselection of patients with mild or uncomplicated pelvic infection, excluding those patients with tuboovarian abscesses or diffuse peritonitis.[7] Such patients are treated best by inpatient parenteral therapy and would be inappropriate candidates for outpatient therapy.

A limitation of this study is that the diagnosis of salpingitis was made by a combination of clinical signs and symptoms (Table I).[22, 23] Unfortunately, the laparoscopic confirmation of the diagnosis of acute salpingitis may occur only in 65% of patients who meet similar clinical criteria.[26] Supportive of the correct diagnosis is the fact that 63% of the subjects had a pathogen isolated from their endocervix and endometrium (or both) commonly associated with acute salpingitis, either *N. gonorrhoeae* or *C. trachomatis*. In addition, 58% of the women had these pathogens isolated from endometrial cultures. Sweet[27] had reported a similar frequency (53%) of upper tract infection with *N. gonorrhoeae* and *C. trachomatis* in 188 women with acute pelvic inflammatory disease.

The criteria used were developed to select women with mild or uncomplicated acute salpingitis, free of severe toxicity, pelvic abscesses or diffuse peritonitis, who would be acceptable candidates for ambulatory therapy, In addition, selecting women with mild acute salpingitis would tend to enroll more subjects with pelvic infection caused by *C. trachomatis*, who often have relatively benign clinical examinations compared with women with gonococcal or nongonococcal, nonchlamydial salpingitis.[28]

Overall the microbiologic response for *N. gonorrhoeae* and *C. trachomatis* was excellent. At days 13 to 15, the gonococcal microbiological response was 100% in both groups. None of the patients in either study group had evidence of a persistent chlamydial infection during the study. One patient in the ofloxacin group had a recurrent gonococcal and chlamydial infection at the 25- to

30-day visit. Although this may be a microbiologic failure, it is more likely a reinfection from an acknowledged untreated sexual partner rather than a relapse.

Despite the clinical and microbiologic evidence of response to the therapeutic regimens administered, questions could arise about adequacy of the complete eradication of a nongonococcal, nonchlamydial infection of the upper genital tract. This issue is of concern because of the limited in vitro activity of ofloxacin and other quinolones against anaerobic bacteria. In one salpingitis study ciprofloxacin failed to eradicate bacterial-vaginosis–associated organisms of the upper genital tract from posttreatment endometrial cultures in women despite excellent clinical response.[29] Whether such persistence of organisms portends a greater risk for infertility, ectopic pregnancy, and other sequelae of salpingitis is unclear.

All the subjects judged to be clinically cured had normal clinical severity scores (value 0 or 1) at their second and third follow-up visits, and their temperature and white blood cell counts returned to within the normal range. These markers are indirect assessments of response to infection and cannot substitute for laparoscopically confirmed[27] response of infection, which was not used in this study. Follow-up of subjects 3 years later demonstrated pregnancies in 54% of the women treated with ofloxacin and in 33% of those in the cefoxitin-doxycycline group. These rates represent a minimum, since the survey was a retrospective, passive medical record review of one institution without correction for voluntary infertility or lack of further contact. The crude rate of intrauterine pregnancy (uncorrected for prior or subsequent salpingitis) for the ofloxacin group is similar to the rate of 63% observed in Westrom[30] in a series of 415 women with previous pelvic inflammatory disease.

In this study a regimen of oral ofloxacin, 400 mg twice daily for 10 days, was of equal efficacy to a regimen of intramuscular cefoxitin, 2 gm, plus oral probenecid, 1 gm, followed by oral doxycycline, 100 mg twice a day for 10 days, in treating patients with uncomplicated acute salpingitis. Both regimens had clinical efficacy of 95% or greater, with eradication of *N. gonorrhoeae* and *C. trachomatis* in every evaluable patient except in one woman who may have been reinfected by an untreated sexual partner. Significant side effects in both groups were mild and similar in nature, with only one severe episode requiring discontinuation of the medication. Although the number of subjects in this study does not permit firm conclusions regarding the equivalence of the two regimens, 400 mg of oral ofloxacin given every 12 hours for 10 days is a promising single-agent treatment for uncomplicated acute salpingitis. If these results are confirmed in larger series, particularly in women with chlamydial infection, single-agent therapy with ofloxacin may offer an acceptable alternative to combination therapy for the outpatient treatment of uncomplicated acute salpingitis.

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The clinical experience with ofloxacin in the treatment of sexually transmitted diseases

Michael L. Corrado, MD
Raritan, New Jersey

Sexually transmitted diseases are an important cause of morbidity in women. Some of the common causes of sexually transmitted disease include *Neisseria gonorrhoeae, Chlamydia trachomatis,* and in some patients *Ureaplasma urealyticum. N. gonorrhoeae* has become more resistant to traditional therapies including penicillin. Resistance to penicillin is mediated by the elaboration of β-lactamase for most organisms and by chromosomal resistance in others. Resistance to tetracycline and spectinomycin has been clearly identified, as has an increase in the minimum inhibitory concentrations to some cephalosporins. Because of the potential for concomitant infection with *N. gonorrhoeae* and *C. trachomatis,* many patients are currently treated with a combination of a single dose of ceftriaxone plus a tetracycline for 7 days. Therefore it is significant that a single agent, ofloxacin, has been introduced recently for the treatment of such mixed infections. It also has the potential for treating *N. gonorrhoeae* resistant to other drugs. (AM J OBSTET GYNECOL 1991;164:1396-9.)

Key words: *N. gonorrhoeae, C. trachomatis,* sexually transmitted diseases, urethritis

In this article we review data from clinical trials conducted in the United States between 1984 and 1988. These data represent a compilation across several multicenter trials, all of which were conducted by the Robert Wood Johnson Pharmaceutical Research Institute. In addition, the world literature from February 1983 to February 1990 will be reviewed.

From the Robert Wood Johnson Pharmaceutical Research Institute. Reprint requests: Michael L. Corrado, MD, Robert Wood Johnson Pharmaceutical Research Institute, Route 202, Raritan, NJ 08869-0602.
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Cervicitis

Ofloxacin was used in the treatment of women with cervicitis. In one study in which ofloxacin was compared with doxycycline, 344 women were enrolled. The regimens included ofloxacin, 300 mg twice daily, versus doxycycline, 100 mg, twice daily, both given for 7 days. In that study a similar percentage of patients receiving ofloxacin had *Neisseria gonorrhoeae* eradicated from various sites as was eradicated with doxycycline (Table I). It is important to note that patients with cervicitis may have simultaneous infection at multiple sites including the anus and pharynx, and as seen in this study, a 7-day course of ofloxacin was effective in eradicating

Table I. Eradication rates of *N. gonorrhoeae* by site*

Site	Regimen		
	Ofloxacin, 300 mg, bid, × 7 days	*Doxycycline, 100 mg, bid, × 7 days*	*Ofloxacin, 400 mg, single dose*
Urethra	1/1	—	10/10
Pharynx	10/10	5/6	8/8
Anus	10/10	11/11	20/20
Cervix	52/53	37/40	90/90

*Studies conducted by and on file at the Robert Wood Johnson Pharmaceutical Research Institute.

N. gonorrhoeae from all patients and all sites with the exception of a single patient in whom the organism persisted in the cervix. In addition, 45 patients who received ofloxacin had concomitant infection with *Chlamydia trachomatis.* Forty-four of 45 patients had their chlamydia infection eradicated. This compares with 39 of 40 patients receiving doxycycline who had the pathogens eradicated. Several other trials involving patients with gonorrhea were conducted in the United States in which the efficacy of ofloxacin was investigated. A total of 1172 isolates of *N. gonorrhoeae* were identified from all clinical trials conducted in the United States. All isolates of *N. gonorrhoeae* were susceptible to ofloxacin. Ninety-nine percent of patients receiving ofloxacin had their infections either cured or improved as did 97% of patients who received comparative regimens. Table I further identifies eradication rates for women enrolled in single-dose studies involving ofloxacin for gonococcal disease. As can be noted, all patients with anal and pharyngeal involvement had the organisms eradicated, as did all 90 patients with cervical involvement. When reviewing the eradication rate from trials conducted in men and women with gonococcal and nongonococcal urethritis or cervicitis, virtually idential eradication rates were identified for *Chlamydia* in both ofloxacin- and doxycycline-treated recipients (Table II).

Pelvic inflammatory disease

Studies conducted in women with pelvic inflammatory disease in the United States in which microbiologic identification was confirmed revealed that all 47 women with pelvic inflammatory disease who had gonococcal disease had the pathogens eradicated (Table III). In addition, 19 had chlamydial pathogens identified, and again, all 19 patients had the *Chlamydia* eradicated. In contrast, although all 23 women with gonococcal disease randomized to treatment with cefoxitin plus doxycycline had their pathogens eradicated, 2 of 15 women treated with the doxycycline regimen had persistence of *Chlamydia.*

Table II. Bacterial eradication rates in multiple-dose studies*

	Ofloxacin	*Doxycycline*
Gonococcus	149/155	176/178
Chlamydia	141/147	167/172

*Studies conducted by and on file at the Robert Wood Johnson Pharmaceutical Research Institute.

Table III. Bacterial eradication rates in pelvic inflammatory disease*

	Cefoxitin + doxycycline	*Ofloxacin*
Gonococcus	23/23	47/47
Chlamydia	13/15	19/19

*Studies conducted by and on file at the Robert Wood Johnson Pharmaceutical Research Institute.

The medical literature from trials conducted outside of the United States revealed 37 published reports from 15 countries involving more than 3600 patients (see Bibliography). Doses of ofloxacin ranged from 300 to 600 mg/day either as a single dose or for as long as 7 days. Although clinical cure and improvement rates varied, by and large most patients had eradication rates of 90% or higher. Microbiologic eradication rates were almost always in the 90% range or higher, with the lowest microbiologic eradication rate occurring in studies conducted in Japan, where in one study only 71% of isolates were eradicated.

Comment

Studies conducted in sexually transmitted diseases suggest that ofloxacin retains a high degree of intrinsic activity against penicillin-resistant as well as penicillin-susceptible gonorrhea. In addition, it exerts a high degree of activity against *Chlamydia,* a pathogen that may be either a copathogen or the sole cause of sexually transmitted disease in some patients. Ofloxacin appeared effective at eradicating pathogens from multiple sites including the anus and pharynx as a single dose for gonorrhea and thereby decreases the likelihood of a potential for disseminated gonococcal disease from a noneffectively treated focus. It seems, however, that the treatment of sexually transmitted diseases involving *Chlamydia* with ofloxacin would require the more traditional 7-day course and not single-dose therapy.

The use of ofloxacin in pelvic inflammatory disease appears to be comparable with the combination of cefoxitin plus doxycycline. Thus ofloxacin offers the potential of monotherapy for the treatment of mixed sexually transmitted infections. The published literature also demonstrates that ofloxacin is effective in gonorrhea and chlamydia, and by and large, studies from across countries have delineated the highly reproduc-

ible eradication rates and clinical cure rates seen in the United States. The few exceptions occurred in Japan and Germany, where a lower dose may be implicated in the lower microbiologic eradication rates.

In conclusion, ofloxacin appears to be effective as monotherapy and single-dose therapy for gonococcal disease, as well as when given twice daily for 7 days for the treatment of mixed infection caused by *C. trachomatis* and the gonococcus.

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Summary

Sebastian Faro, MD, PhD
Houston, Texas

The quinolones are a unique class of antimicrobial agents in that they affect the cell's capability to reproduce by interfering with deoxyribonucleic acid (DNA) synthesis. The bacterial enzyme DNA gyrase is essential for DNA replication. All organisms contain a group of enzymes known as DNA topoisomerases, to which DNA gyrase belongs. However, only the bacterial DNA gyrase inserts negative supercoils into DNA.

Coiling of bacterial DNA is essential since the bacterial chromosome is composed of double-stranded DNA 1300 μm long, and it must fit into a bacterium whose average size is 2 μm long and 1 μm wide. Thus DNA gyrase or topoisomerase II causes nicks in the double-stranded DNA, producing negative supercoils, and then seals the nicked DNA. The quinolone's ability to affect DNA gyrase, which is found only in the bacterial cell, may explain why these agents can interfere with bacterial DNA synthesis and not mammalian DNA activity.

The progenitor of the quinolones is nalidixic acid with activity limited to gram-negative facultative anaerobes. The quinolones were slow to develop until the introduction of fluorine and piperazine into the basic molecule. The new quinolones were found to be active against gram-positive, gram-negative activity, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*. There are many quinolones, three of which are approved (ciprofloxacin, norfloxacin, and ofloxacin), and some of which are not available: nalidixic acid, flumequine, cinoxacin, oxolinic acid, pipemidic acid, piromidic acid, rosoxacin, enoxacin, fleroxacin, lomefloxacin, amifloxacin, and difloxacin.

The spectrum of activity of the newer quinolones has evolved to a significant degree that these agents are effective for the treatment of a variety of infections. In considering the agents that are currently available, norfloxacin (Noroxin), ciprofloxacin (Cipro), and ofloxacin (Floxin) are effective against gram-negative facultative anaerobes, such as *Pseudomonas, Campylobacter, Citrobacter, Providencia, Hafnia, Escherichia, Klebsiella, Proteus, Serratia,* and *Enterobacter*. These agents are also active against *Haemophilus influenzae, Branhamella (Moraxella), Acinetobacter, Aeromonas,* and *Neisseria gonorrhoeae*.

The new quinolones are less active against the gram-positive aerobes than against the gram-negative aerobes. However, among the gram-positive bacteria, the quinolones are more active against staphylococci than streptococci. Ciprofloxacin and ofloxacin are active against *S. aureus,* coagulase-negative staphylococci, and *Staphylococcus saprophyticus*. Ofloxacin and ciprofloxacin show good activity against *Streptococcus pneumoniae,* β- and nonhemolytic streptococci, β-hemolytic streptococci (Lancefield groups A, B, C, and G), and *Enterococcus,* although ciprofloxacin is more active than ofloxacin against the β- and nonhemolytic streptococcus and *Enterococcus*.

The quinolones are not very active, in general, against *Gardnerella vaginalis* and anaerobes. However, ofloxacin is more active than ciprofloxacin against members of the *Bacteroides fragilis* and *B. melaninogenicus/oralis* groups. Fusobacteria tend to be resistant to ofloxacin and ciprofloxacin, whereas *Mobiluncus* tends to be very sensitive to both of these quinolones. The gram-positive obligate anaerobes tend to be resistant to ofloxacin with peptostreptococci more susceptible to ciprofloxacin. The Clostridia are resistant to both ofloxacin and ciprofloxacin.

Both ofloxacin and ciprofloxacin have activity against *Chlamydia trachomatis*. Neither of these quinolones is particularly active against *Mycoplasma* or *Ureaplasma*.

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Thus the quinolones, particularly ofloxacin, have a broad spectrum of activity that makes the antibiotic well suited for the gynecologic patient. The quinolones should not be used in pregnant and breast-feeding patients because of the potential to cause arthropathy in children. However, in the postpartum patient who is not breast feeding, the quinolones may have a role.

Female genital tract infections are either caused by sexually transmitted organisms or the patient's own endogenous microflora of the genital tract. The two most common sexually transmitted bacteria are *N. gonorrhoeae* and *C. trachomatis,* both of which may cause limited or significant soft tissue infection. These two bacteria can cause urethritis, cervicitis, or endometritis, as well as the more serious salpingitis. Early acute salpingitis is most likely caused by *N. gonorrhoeae,* with fewer cases caused by *C. trachomatis.* Ofloxacin is an ideal agent for the treatment of these infections, since a single agent can be used effectively to treat infection caused by both these organisms. This agent is particularly useful, since the β-lactamase–producing and tetracycline-resistant strains of *N. gonorrhoeae* are not resistant to ofloxacin. The concern of polymicrobial nongonococcal, nonchlamydial salpingitis would not be of particular concern in patients with early acute salpingitis.

N. gonorrhoeae is responsible for 60% to 80% of the cases of acute salpingitis in the United States; *C. trachomatis* is responsible for 20% to 25% of the cases. The aerobic and anaerobic bacteria are probably not involved in early acute salpingitis. These bacteria play a significant role in these patients in whom peritonitis and inflammation of the bowel have become a significant part of the infectious process. In these patients use of ofloxacin or another quinolone without the concomitant use of an anaerobic agent would be inappropriate. This distinction might be made by obtaining an endocervical specimen for Gram's stain and rapid monofluorescent antibody testing for the detection of *Chlamydia.*

A Gram's stain that reveals the presence of intracellular diplococci can be taken as presumptive evidence for the presence of *N. gonorrhoeae.* A positive fluorescent antibody for *Chlamydia* can be viewed as indicative of infection. The presence of either or both of these bacteria does not rule out the presence of other bacteria, but it is unlikely. In any event, treatment for salpingitis may be carried out with ofloxacin or a combination of clindamycin or metronidazole plus ofloxacin, thus broad coverage is provided for the anaerobes, and ofloxacin provides good gram-negative and fair gram-positive coverage.

Soft tissue infections, such as postpartum endometritis and pelvic cellulitis after gynecologic surgery, may also be treated by using a combination of clindamycin or metronidazole plus ofloxacin. These combinations also offer the advantage of oral administration. Parenteral ofloxacin is not yet available. The use of these orally administered agents will provide more than adequate tissue levels. Oral administration also allows the patient to continue therapy as an outpatient once the acute symptoms and signs of infection have resolved.

Finally, it is well known that the excellent activity of the quinolones against gram-negative facultative bacteria makes them ideal for the treatment of urinary tract infection. Perhaps these agents should be used in patients who might harbor resistant bacteria, such as those with repeated infection, in the presence of indwelling catheters and exposure to antibiotics.

Thus the quinolones, and ofloxacin in particular, provide the physician with a unique opportunity for the treatment of bacterial infections. The ability to use one agent for the treatment of both *N. gonorrhoeae* and *C. trachomatis* facilitates compliance on the patient's part and reduces cost and therefore may serve as a potential replacement for ceftriaxone and doxycycline. Ofloxacin is an excellent agent for the treatment of urinary tract infections caused by gram-negative facultative anaerobes as well as *S. saprophyticus.* The mechanism of action of the quinolones reduces the potential for significant toxic effects. Adverse reactions to the quinolones are rare and include nausea, vomiting, diarrhea, rash, pruritus, dizziness, headache, and insomnia. The most frequent side effects are gastrointestinal.

Although the quinolones are broad-spectrum agents potentially suited for a variety of infections, the physician must establish the correct microbiologic diagnosis. The physician must understand the spectrum of activity of the quinolones and realize that all quinolones are not equal in their activity against all bacteria. It is important that the patient be reevaluated during therapy to determine if the antibiotic being used is effective and the signs and symptoms of infections are resolving. Patients infected with *N. gonorrhoeae* and/or *C. trachomatis* should be recultured approximately 2 weeks after the completion of therapy to establish that the organism has been eradicated.